Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii

TROPICAL AND SUBTROPICAL AGRICULTURAL RESEARCH

Under PL 89-106, Special Research Grants

Progress and Achievements
The Pacific Basin Group
1989

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On the Cover

The larva of *Pareuchaetes pseudoinsulata*, a natural enemy of *Chromolaena odorata*. (See the report "Biological Control of the Weed *Chromolaena odorata*" on page 16.)

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ACKNOWLEDGMENT

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FOREWORD

The program for tropical and subtropical research in the Pacific Basin supports research on the organisms, environments, and processes of tropical agricultural systems of the American-affiliated Pacific areas. Its principal purpose is to strengthen the research capacity of the Pacific Basin institutions and to facilitate the transfer and use of results. Emphasis is on high quality agricultural research conducted by institutions in the region having demonstrated research capabilities. Through collaborative research efforts, the program focuses primarily on overcoming the most critical constraints to crop production, processing, and marketing.

The program provides assistance to domestic agriculture in many ways. These include research on tropical and subtropical crops presently grown in the U.S., alternative crops, control of threatening pests, enhancement of the genetic stocks for many major U.S. crops, protection and conservation of soil and water resources, and the development of technologies important to the small farmer. The primary emphasis is on improving small-scale agricultural systems of food and feed production. Additionally, a large portion of the research is conducted on low-input agriculture strategies, including more effective non-chemical means for controlling pests and diseases, new uses for farm products, and innovative management and marketing concepts.

In summary, research conducted under the Special Grant provides agricultural and nutritional benefits by increasing productivity of small farms in the tropical areas of the American Pacific. It develops our reservoir of knowledge for the special needs associated with tropical and subtropical agriculture. Further, the research results help to insure that U.S. businesses will be able to share in the large and growing market for agricultural services and equipment in the developing world of the tropics.

This report is published with the purpose of disseminating recent information on achievements and trends of agricultural research activities conducted under the Special Grant for Tropical and Subtropical Agriculture. It summarizes and interprets the results and progress of a selected group of these investigations. All projects deal with high priority areas which should result in solutions to critical problems, as well as develop new opportunities for agricultural production and marketing in the tropics of the Pacific Basin.

M. Ray Smith
Pacific Basin Coordinator

PROJECT ACHIEVEMENTS
SECTION 406

Intercrop Modeling: A Generalized, Process-level Approach

Principal Investigator:
R. Caldwell, Dept. of Agronomy & Soil Science, UH

Nature of Project

Intercropping—the simultaneous production of more than one crop species in the same field—is a key feature of farming in much of the tropics. Farmers benefit from intercropping through more efficient use of land and through reductions in pest and disease problems.

Intercrops are complex. While a sole (single) crop usually has a limited range of recommended management options, there are limitless possible optima for managing intercrops. "Average" recommendations are not adequate.

The effectiveness of a management decision depends strongly on the farmers' particular physical environment (including weather). Of even greater importance is the diversity in goals that farmers have for their intercrops. These goals are influenced by the social environment and the personal value of each species in the intercrop. To meet his goal, the farmer must shift resources among the species by managing interspecific competition. The best means of managing competition is not usually known beforehand and will depend on the environment.

The Intercrop Modeling project is working to provide insight into crop management, and to aid the transfer of technology to resource-poor farmers in the tropics. Computer simulations are being developed to evaluate large numbers of intercrops, management packages, and farm environments— more than could ever be duplicated in field trials. In order to develop the simulations, a number of basic research questions must be answered: How do species grow and compete with each other in intercrops? How can we represent complex agricultural systems so that the intercrop model will have general applicability in the tropics?

Major Achievements

A combination of field equipment, computer hardware, and computer software has been developed for measuring competition for light in intercrops. The system employs video image analysis and will be used to quantify parameters needed in the intercrop model. We have also produced a prototype systems simulation entitled "AGSYSTEM: An Object-Oriented Model of the Agricultural Systems Hierarchy" (Figure 1). AGSYSTEM

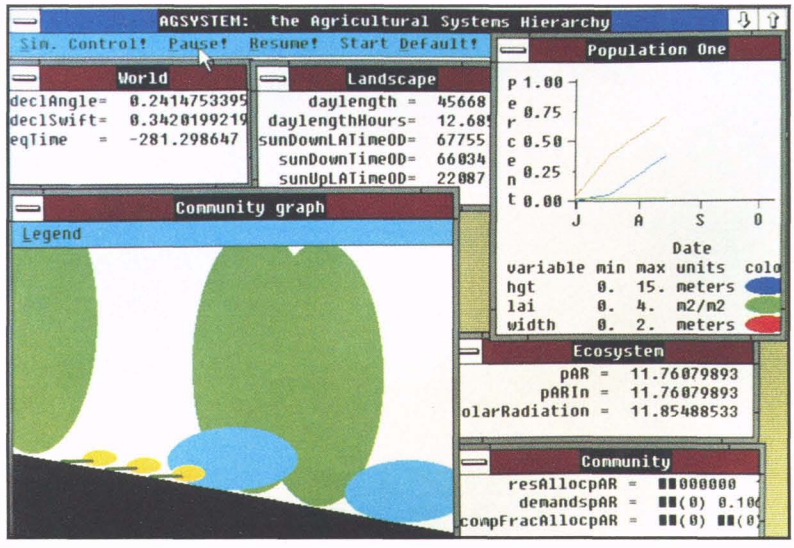


Figure 1. Example screen from "AGSYSTEM: An Object-Oriented Model of the Agricultural Systems Hierarchy." Results for different levels in the hierarchy are displayed in their own window. The screen indicates the current status of the landscape level (weather information), community level (competition between corn and soybean for light), and the population level (corn has finished its growth and soybean, which emerged on March 6, is continuing to grow).

is a microcomputer-based program that lays a foundation for complex systems simulations. It has special capabilities for simulating multiple farms on a landscape, multiple agroecosystems on a farm, and multiple species within each agroecosystem. Results from AGSYSTEM are displayed through a graphical user interface (Microsoft® Windows). These capabilities were implemented with a new-generation programming approach—object oriented programming—that helped organize and generalize the simulation. AGSYSTEM includes a new model of competition for light in intercrops (Figure 2).

Importance to Users

AGSYSTEM is currently available as a teaching tool. Students and scientists can experiment with a number of factors and evaluate their impact on competition for light. Relative planting date, row spacing, row arrangement, and plant size and leafiness can be studied by the user.

Object-oriented programming, the generalized simulation structure, and the graphical user interface represented by AGSYSTEM may be long-term contributions to modeling and agrotechnology transfer in the tropics.

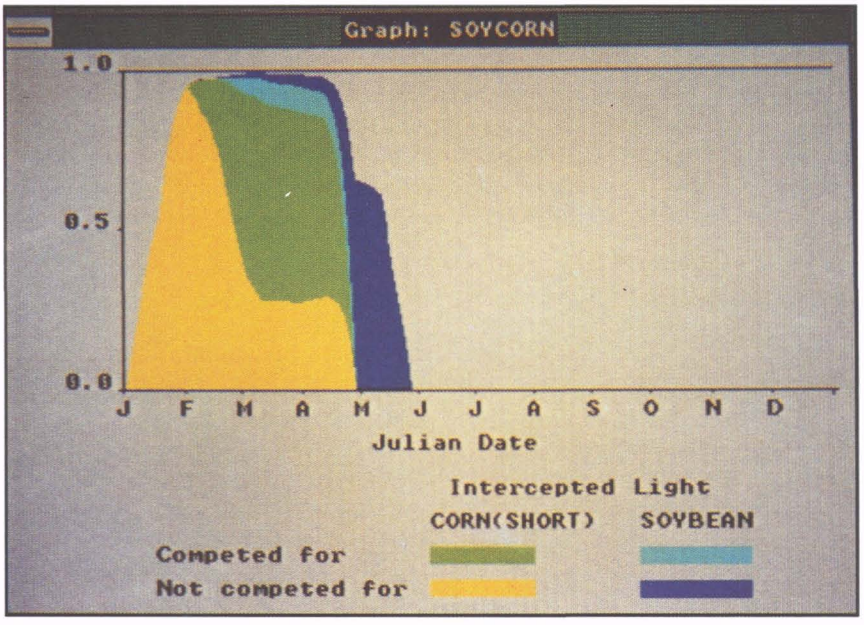


Figure 2. Detail of graph window for a corn/soybean intercrop community simulated by AGSYSTEM. The window illustrates interspecific competition for light. The yellow plus green region represents the fraction of incoming light intercepted by corn. Cyan plus dark blue is the fraction for soybean. Of the amount intercepted, some could have been intercepted by either population. That is the light subject to competition (green plus cyan). The corn was taller than the soybean and so was more competitive (green fraction greater than the cyan fraction). Note that there was no interspecific competition before the soybean emerged (Feb. 2) or after the corn was harvested (April 29).

Sap Analysis as a Guide to Efficient Fertilization of Tropical Vegetable Crops

Principal Investigator:
R. R. Coltman, Dept. of Horticulture, UH

Nature of Project

Nitrogen (N) is one of the most difficult fertilizer nutrients to manage properly because of the roles that microorganisms and weather play in determining N availability. Because of the unpredictability of these factors, a quick, field-administered test for N sufficiency in plant tissues during crop development would be useful.

Major Achievements

This project has demonstrated that nitrate levels in tomato, pepper, and cucumber petioles are sensitive indicators of the N status of these crops, and that fairly simple sap nitrate tests are sufficiently sensitive to indicate whether N fertilization is needed. Minimum levels of sap nitrate associated with maximum yields of field-grown tomatoes, peppers, and cucumbers have been identified. A

management strategy for N fertilization of greenhouse-grown tomatoes has been developed and verified (Figure 3). Documentation of these achievements has begun to reach the public through numerous publications.

The development of a strategy for managing N fertilization of greenhouse tomatoes is the most advanced achievement of this research. Several of the largest tomato growers in Hawaii have expressed a desire to begin using the strategy. These growers also would like to have quick test methodologies for managing other nutrients developed, and widespread management of fertilization based on monitoring with quick tests probably will be restricted until a more comprehensive testing program can be developed. Encouraging preliminary results with quick testing for potassium in tomatoes suggest that multinutrient management is feasible if additional efforts are made.

Importance to Users

Presentation of the results of this project in national and international circles has resulted in considerable interest in—and encouragement for—further work. One researcher in New Zealand has incorporated some of the findings from this project into his work in quick testing with fruit and vegetable crops. The use of quick testing methodologies has particularly great appeal in the tropics, where sophisticated analytical test facilities are generally nonexistent. Avenues are currently being explored to develop a network of international researchers to pursue quick testing approaches for fertilization management.

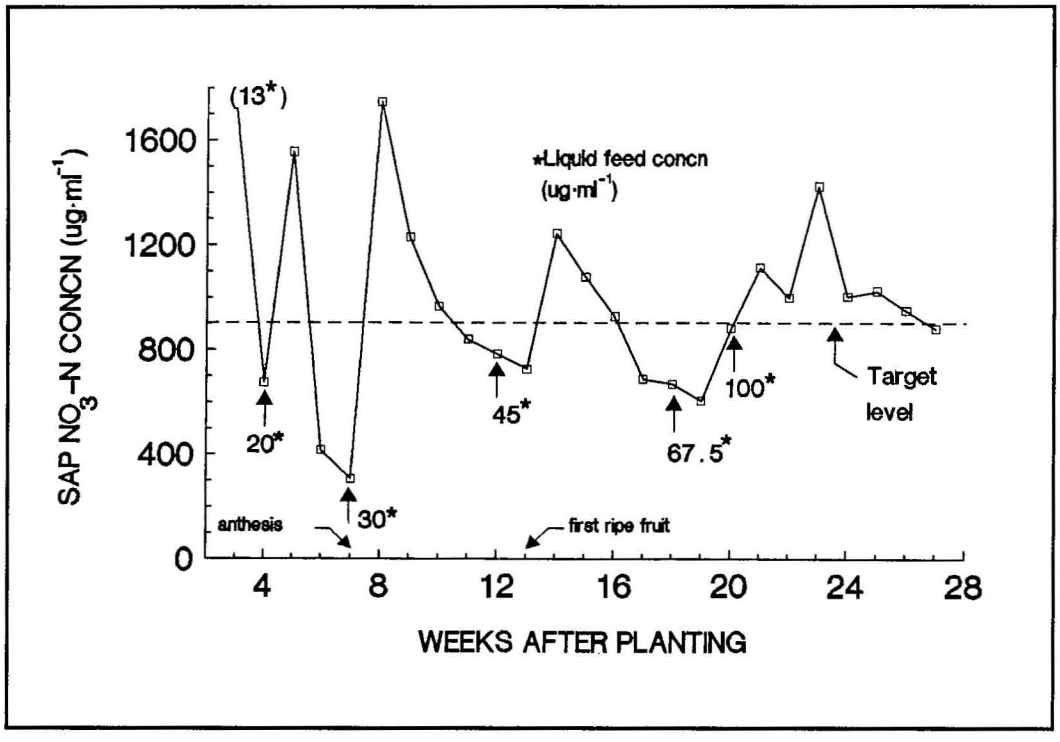


Figure 3. Sap NO_3–N in maximally yielding greenhouse tomatoes.

Potential of Potato Production in Guam, Saipan, and Other Micronesian Islands

Principal Investigators:
M. Marutani, University of Guam
J. Demeterio, University of Guam
R. Muniappan, University of Guam

Nature of Project

Consumption of potatoes in Guam and other Micronesian islands has increased in recent years and currently over a million pounds of potatoes are consumed per year in Guam. Recent studies on potato production in the hot tropical climates of Southeast Asia have shown a potential for production of potatoes in the Pacific.

Major Achievements

With the collaboration of the International Potato Center, screening of cultivars of *Solanum* spp. (L.) has been conducted on the islands of Guam and Saipan during the dry season. Principal criteria for evaluation included yield, emergence (%), harvest (%), marketable tubers (%), average tuber weight, number of tubers per plant, number

of stems per plant, and disease and pest resistance. In general, yield was low. Results of 11 field experiments during 1982–1987 showed the best yield obtained was 29.2 t/ha of Kennebec in the trial on Guam 1985–86. Two other cultivars, Red Pontiac and Sequoia, yielded 27.1 t/ha. Among the cultivars that were developed recently, CIP720088, MS-35.22 and DT0-28 produced higher yield than others. Plant development was very rapid in tropical conditions. Emergence was observed around 10 days after planting (DAP) for most cultivars and tuberization occurred around 30 DAP, which was much earlier than in temperate regions. The short-day condition cancels out the adverse effect of high temperature in delaying tuberization. Canopy cover reached the maximum level at around 50–55 DAP. The harvest date was 75–85 DAP.

Various disease and pest problems occurred during the experiments. Early blight (*Alternalria solani*) and stem rot (*Sclerotium rolfsii*) were major fungal diseases in Guam. One of the major disease problems in Saipan was *Rhizoctonia solani*. More than 50 percent of Kennebec and Sequoia were infested by the organism after a typhoon hit the island during the 1986–1987 trial. Red Pontiac was infested less than Kennebec and Sequoia. The incidence of tuber rot by soil-borne bacteria (*Erwinia* spp.) and nematode infestation (*Meloidogyne* spp.) significantly reduced the production of marketable tubers in some fields. High soil temperature (25–30°C) promoted the occurrence of soil-borne diseases. Red Pontiac was especially susceptible to *Meloidogyne* spp. Insect problems varied from year to year and from location to location. The major pests found in Guam were leaf miner and mealy bugs. Without regular application of insecticides, development of plant canopy was significantly reduced.

In addition to varietal trials, agronomic experiments were conducted to obtain information on cultural practices for growing potatoes in the tropics. A fertilizer experiment was done to compare the effectiveness of using green manure, *Crotalaria juncea*, L. with application of supplemental N fertilizer. Highest yield of Kennebec was obtained with the combination of incorporating green manure prior to planting and application of additional N fertilizer at 25 and 55 DAP (Figure 4). Application of additional N fertilizer was essential to having good yield. Use of green manure would increase tuber yield slightly.

Effects of supplemental irrigation on plant development of the cultivar Red Pontiac were determined. The optimal water requirement was 5.5 mm/day. Frequent irrigation promoted rapid canopy development. In contrast, nonirrigated plants showed reduced canopy growth. The heavily irrigated plants had reduced yields primarily due to outbreaks of root knot nematodes (*Meloidogyne* spp.) and soft rot bacteria (*Erwinia* spp.).

Importance to Users

Heat-tolerant cultivars suitable to the tropical climate of islands are not available yet. Proper cultural practices are essential in order to obtain good yield. Planting seed tubers from November to December is recommended because the short day condition cancels out the adverse effect of high temperature in delaying tuberization. Supplemental application of N fertilizer and irrigation are necessary for plants to have vigorous growth and to obtain high yield. Weekly spray of fungicide is recommended. Stem rot (*Sclerotium rolfsii*), the most common soil-borne fungal disease in Guam, can be controlled by drenching fields with fungicides. Although there are many obstacles for farmers to overcome, there is a potential, with care, to grow potatoes on Pacific islands.

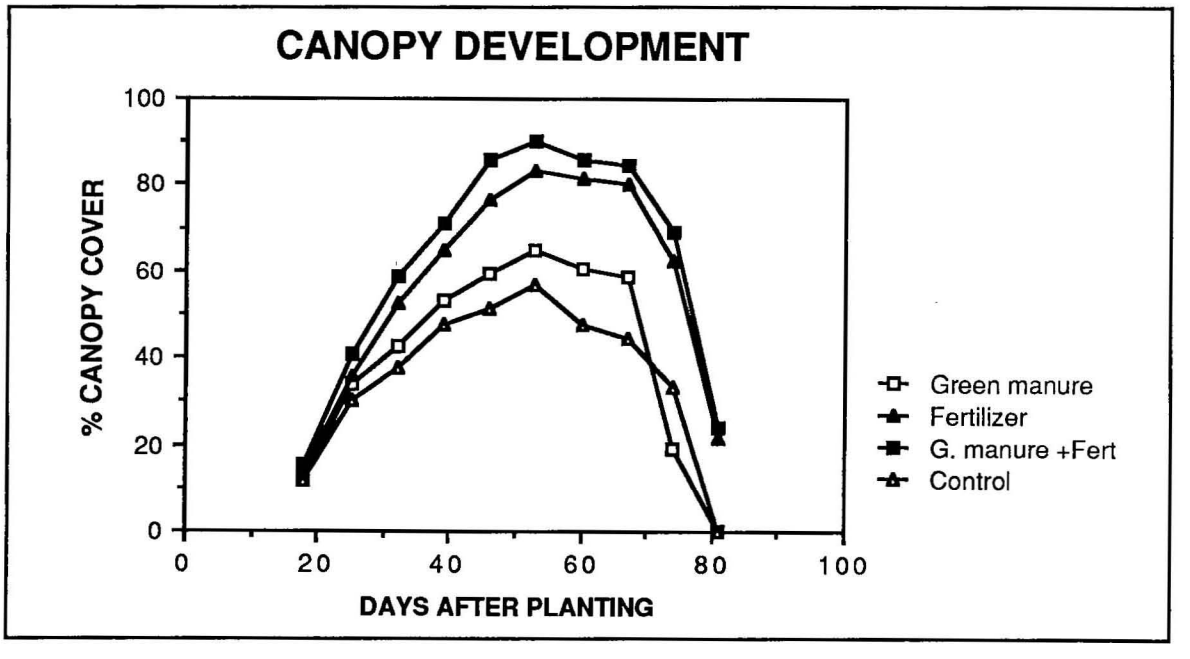


Figure 4. Canopy development of cultivar Kennebec as influenced by the application of green manure (*Crotalaria juncea* L.) and fertilizer.

Environmental Factors Affecting Flowering in Some Vanda and Dendrobium Hybrids in the Tropics

Principal Investigator:
J. McConnell, University of Guam

Nature of Project

Flower production in orchids varies from season to season in the subtropics and tropics. This variation is not consistent from year to year, due to an interaction between the plants and the environment. The primary environmental factors affecting flowering have not been clearly identified. The objective of this project is to determine which environmental factors affect flowering in vanda and dendrobium hybrids.

Major Achievements

The flowering behavior of several orchid hybrids is being monitored concurrently with the recording of weather data at several locations in Guam. Phenological data are being recorded for different aged vandas and dendrobiums. The weather factors recorded and logged at 15 minute intervals are solar radiation, rainfall, air temperature, wind speed, and wind direction. The data are uploaded to a personal computer where they are accumulated, summarized and archived. A database manager is being developed to accumulate and summarize the data. The program can communicate directly with the loggers and has error-checking capabilities. The program will also alert us to data that have not been entered.

Importance to Users

Preliminary data indicate that, as expected, solar radiation has a significant effect on flowering; however, different orchid cultivars have flowered at different times of the year. Also, dendrobiums growing in 30 percent shade showed peak flowering one month before the dendrobiums growing in full sun. The greatest flowering occurred in January and February. This information on flowering behavior will be useful in the future for developing new cultivars for commercial production.

Prediction of Maximum Yields of Sugarcane with Growth Simulation Models

Principal Investigators:
J. A. Silva, Dept. of Agronomy & Soil Science, UH
M. M. Anders, Dept. of Agronomy & Soil Science, UH

Nature of Project

The project was designed to provide detailed growth data for sugarcane throughout its growth period to allow for the development of a preliminary growth simulation model. The management inputs of nitrogen and irrigation were varied to measure their effect on growth and also to determine the levels required to achieve the maximum dry matter and sugar yield. The effects of nitrogen and irrigation on dry matter and sugar yield have been determined while the preliminary growth simulation model is being developed from the basic equations of the growth processes.

Major Achievements

A field experiment with 6 rates of N (0, 224, 364, 504, 729, and 953 kg/ha) and 2 levels of irrigation (1.2 and 2.4 times evaporation from a class A pan) was conducted with sugarcane cultivar H65-7052 (*Saccharum* species hybrid) on a Cumulic Haplustoll in Hawaii to measure yield and tissue N response. The highest dry matter and sugar yields (119 and 50 Mg/ha, respectively) were produced with 364 kg N/ha when the crop was harvested at 26 months. The lack of yield response to irrigation indicated that the lowest rate was at least adequate. The high rate of irrigation, 2.4 times pan evaporation, resulted in lower plant N uptake, which suggests that applied N may have been lost by leaching or denitrification. These results confirmed the N rates currently used by the Hawaiian Commercial and Sugar Company where the experiment was conducted.

Crop log (leaf blade) N provided a general guide to the N status of the plant, but primary stalk N reflected the N treatments more closely and was a better indicator of the N status of the plant. Total N uptake in the live, aerial plant was accurately predicted using an exponential growth equation for those treatments receiving N. Predicted N uptake indicated that 90 percent of maximum plant N was taken up by about 11 months in N-fertilized treatments. This was before one-half to three-fourths of the total treatment N had been applied. Nitrogen applications made when the cane was 11 and 12 months old were not reflected in the N concentration of any of the tissues or in dry matter production. It appeared that early applications of N in amounts less than about 70 kg/ha increased the efficiency of N uptake. Sugar plantations in Hawaii have generally made the last N application by the time the cane is 12 months old. Recently, there has been a move to apply the last N earlier, at about 9 months. This research confirms this change and also indicates that the high amounts of N sometimes applied in a single application are inefficient and result in substantial N losses.

An attempt was made to account for the applied N when the crop was harvested. Nitrogen remaining in the live

above-ground parts of the plant was determined, and that in the attached dry leaves and leaf litter was measured before and after burning the crop prior to harvest. Ammonium- and nitrate-N remaining in the soil profile to a depth of 1.5 m were determined following harvest and the amount of N in cane roots was estimated. It was found that about 260 kg of mineralized N/ha was taken up by the 0 N treatment. A N balance was developed which revealed that the amount of applied N recovered varied with N treatment. The N recovered was 0, 34, 32, 29, 24, and 23 percent of the 0, 224, 364, 504, 729, and 953 kg N applied per ha. The 364 kg N/ha rate, which produced maximum cane and sugar yields, had the second highest recovery of applied N and thus relatively high efficiency of N utilization which reduced possible pollution from N leaching into the ground water.

Importance to Users

This research demonstrated that the rate of N used by the plantation on which the experiment was conducted gave the maximum yield with the greatest efficiency of applied N. It also indicated that N applications of 70 kg/ha or less when the cane was less than 11 months old were the most efficient and resulted in the smallest losses of applied N. It demonstrated that the high level of irrigation (2.4 times evaporation from a class A pan) resulted in greater loss of applied N, which could result in greater pollution of the ground water. Plantations generally irrigate at the rate of 0.8 to 1.0 of evaporation from a class A pan so N pollution is expected to be minimal.

Another phase of the project is concerned with developing growth simulation models to predict yields of sugarcane. Basic equations have been developed for the various growth processes of sugarcane and present sugarcane growth and yield response to nitrogen, irrigation, and environment. Work is being done to combine these growth process equations in a preliminary yield prediction equation.

PLANT DISEASE AND PEST MANAGEMENT

Integrated Control of Legume Root-rots Caused by *Rhizoctonia solani*

Principal Investigator:
M. Aragaki, Dept. of Plant Pathology, UH

Nature of Project

Rhizoctonia solani is one of the most serious plant pathogens, particularly as a crown- or root-rotting organism. It can also cause blights of above-ground parts of plants. Although the fungus is commonly encountered and presumably well known among plant pathologists, other fungi have been mistaken for *R. solani*. These other fungi have vegetative characteristics similar to *R. solani*, but in the absence of the sexual stages, they can be distinguished only on the basis of nuclear numbers in vegetative cells. *Rhizoctonia solani* have multinucleate cells, whereas the fungi resembling *R. solani* have vegetative cells with 2 nuclei. Moreover, they do not have appropriate names but are called binucleate, *R. solani*–like or BRS-fungi.

Major Achievements

Rhizoctonia solani and BRS-fungi are frequently found together and isolated from the same root rots. In general, *R. solani* isolates are pathogenic, whereas BRS-fungi are frequently avirulent or weakly pathogenic. Of 37 BRS-fungi tested for pathogenicity, none approached the virulence of *R. solani*, but 16 were found to be sufficiently pathogenic to be unsuitable to apply as biocontrol agents (Figure 5). Twenty-one (21) isolates were determined to be weakly virulent to alfalfa seedlings.

Nine of the latter group of BRS-fungi were tested for their potential to suppress alfalfa seedling blight caused by *R. solani*. The resulting stands for healthy alfalfa varied from 1- to 10-percent in contrast to complete kill of control seedlings. This modest level of protection is seen as encouraging, and significant improvement is anticipated when pathogen inoculum is adjusted to less than overwhelming levels.

Of 13 fungicides examined against vegetative growth of 20 isolates of *R. solani*, 5 were selected as being very inhibitory. Sixty-six (66) isolates of BRS-fungi were tested for their tolerance to these 5 fungicides. None of the 66 isolates tolerated benodanil or carboxin, so these fungicides were temporarily set aside as unsuitable for biocontrol purposes. Twenty-one (21) isolates tolerated at least one of the remaining 3 fungicides, which are triflumizole, chloroneb, and pencycuron. Tolerance to these fungicides was arbitrarily determined at 3 ppm of active substance when vegetative growth was 50 percent or more of control colonies.

Importance to Users

The prognosis of biocontrol of soil-borne diseases caused by *R. solani* utilizing BRS-fungi is very promising. The application of a suitable fungicide should at least have additive effects, but favorable synergistic effects are anticipated. A successful integrated control system could also be applied on other crops affected by *R. solani*.

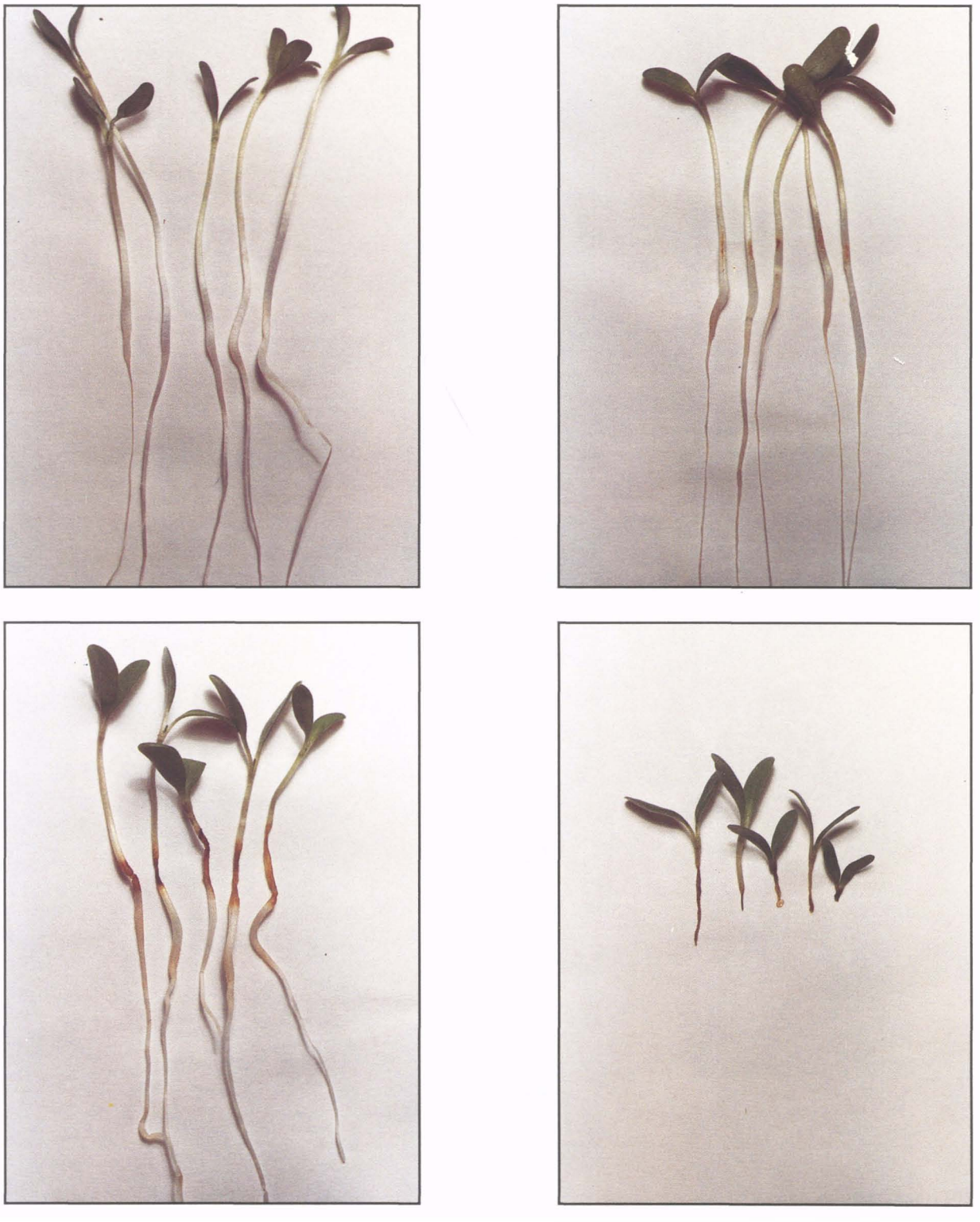


Figure 5. Alfalfa seedlings inoculated with *Rhizoctonia solani* or binucleate, *R. solani*–like (BRS) fungi.

Upper left: Healthy controls
Upper right: Small lesions caused by weakly virulent BRS-fungus
Lower left: Severe infection caused by a strongly pathogenic BRS-fungus
Lower right: Complete rot of hypocotyl and radicle by *Rhizoctonia solani*

Characterization of Living Mulches for Nematode Suppression in Tropical Cropping Systems

Principal Investigator:
J. DeFrank, Dept. of Horticulture, UH

Nature of Project

Screening ground covers for activity on nematodes (in this case reniform) requires a sustainable supply of test organisms for bioassay. In this project, a system has been perfected to produce large quantities of the reniform nematode *Rotylenchulus reniformis* in a sterile culture. This technique will allow us and other researchers to evaluate the impact of naturally occurring plant compounds and biological agents on reniform growth and development under controlled conditions.

Major Achievements

Laboratory assays and greenhouse studies have confirmed preliminary indications that marigold (*T. patula* cv. French Dwarf Sparky Mix) and rhodes grass (*Chloris gayana* cv. Katambora) are not hosts for reniform nematode and can reduce populations to a greater degree than a no-plant fallow.

Sunhemp (*Crotalaria juncea* L. cv. Tropic Sun) is a popular legume cover used in Hawaii. Research has revealed that the reniform nematode can penetrate the roots of this plant, but that egg masses do not appear after the usual period of adult feeding. Sunhemp may be preventing egg mass formation, thus reducing populations of reniform while adding nitrogen and organic materials to the soil.

'Sparky' marigold, 'Katambora' rhodes grass and 'Tropic Sun' sunhemp can be used as rotational green manures without increasing populations of reniform nematode (Figure 6).

Importance to Users

The importance of this research is the scientific confirmation that rotational crops can be an important and effective management tool for reducing populations of a parasitic nematode. This research can provide the basis for other research projects that need to address the biological control of nematodes and other soil-borne crop pests.

The availability and effectiveness of synthetic pesticides have masked the importance of crop rotation in commercial farming operations. As the cost of pesticide registration rises, and as more products are removed from the market, farmers may find themselves unable to produce a commercial crop. As a public institution charged with the stewardship of our agricultural community, it is essential that our research provide a rational basis for selecting rotational plant species to achieve a level of pest control.

In the past, crop rotations referred to corn followed by beans followed by tomatoes. With analytical methods, we can isolate and identify biologically active compounds from plants. With this capability, we can choose rotation crops for the content and potency of chemical compounds which can prevent the build-up of crop pests. Reduced reliance upon synthetic pesticides will require farmers to sacrifice a portion of the growing season to raise a "pest reducing" crop. Researchers in the area of crop protection will need to provide scientific reasons for selecting a pest-reducing rotational crop based on findings similar to the ones obtained in this project.

A future recommendation for lettuce production in Hawaii may sound something like this: After 6 months of lettuce production, rotate with a solid planting of "Sparky" marigold for at least 6 weeks. Inoculate the marigolds with a thrip predator after 3 weeks. Check pheromone traps at regular intervals to determine minimum threshold levels of thrips before a replanting. In this scenario, the ground cover provides a habitat for the predator (to reduce the thrip population) while reducing nematode populations. Obviously, an increased level of on-farm management skill will be required to reduce our dependence on synthetic pesticides.

This research project is just the beginning of an overall scheme to reduce crop pests through crop rotation. It also provides firm scientific evidence for continued and more coordinated efforts in this area.

Figure 6. Bell peppers planted into plastic and organic mulches selected to reduce parasitic nematode populations

Characterization of the Tomato Spotted Wilt Virus Genome

Principal Investigator:
T. German, Dept. of Plant Pathology, UH

Nature of Project

This project is developing an important base of knowledge with which to attack a very serious economic problem of tropical agriculture caused by losses from tomato spotted wilt virus (TSWV) on a large number of crop species. Our work involves the molecular cloning of cDNA copies of the RNA genome of this virus. This was difficult to accomplish because it is not possible to obtain a highly purified preparation of virus to use as starting material.

Major Achievements

The project developed a novel approach using a differential screening technique to obtain a library of cDNA clones, which represents most of the viral genome. In the future we hope to search the library for the sequence which encodes the viral coat protein gene. This information can then be used to construct transgenic plants using currently available techniques of genetic engineering. We predict that these "engineered plants" will have an inheritable resistance to TSWV. This later phase of the work is the subject of our next grant request, and the basic information essential to this important future work (and probably for obtaining funding) was obtained from our present grant. We would like to emphasize the value of funding basic research as a first step towards solving important agricultural problems in the "real world."

Importance to Users

A more immediate application of our current research has been the development of a very rapid and sensitive diagnostic procedure for TSWV. By using the cDNA from the library, we can make radioactive probes which can be used to detect virus in all of the natural hosts of this disease (Figure 7). This is important because it provides a confirming test to the one formerly used and can be used in certain instances where the old test was unreliable. In addition, the test is so sensitive that we can detect virus in small numbers of tiny insects (thrips) which transmit the disease in the field. We will use this test to develop information on the number and distribution of insects carrying viruses in susceptible crop growing areas. Using these tools, we can work with other researchers to manage the use of insecticides for better cost effectiveness and pollution control, to develop resistant varieties and to devise improved management strategies.

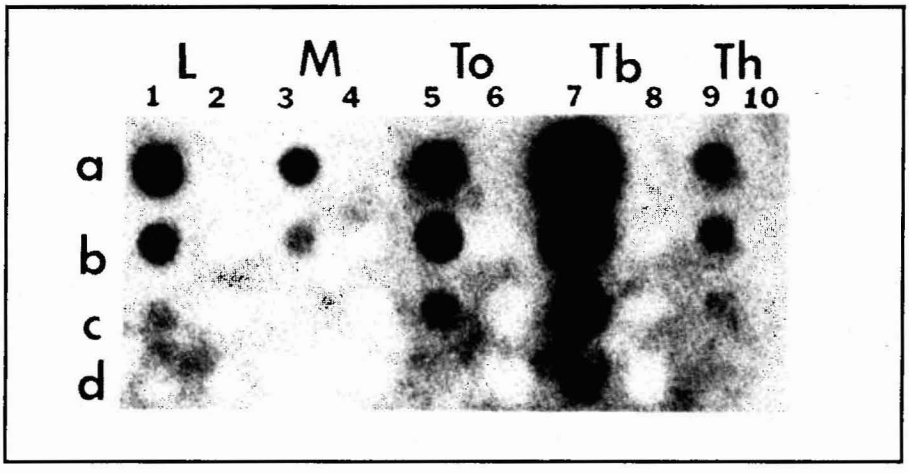


Figure 7. Dot blot assay using a radioactive probe prepared from cloned cDNA of tomato spotted wilt virus. Liquid extruded from test samples was spotted on a special type of paper and exposed to the probe under conditions that would allow the cDNA to hybridize with viral RNA present in infected material. The paper is placed next to X-ray film, which is exposed if the probe has stuck to viral RNA present in the test material. The film is developed and the resulting autoradiogram shows that infected samples (dark spots) can be clearly distinguished from uninfected samples (clear columns) and that the signal produced is proportional to the concentration of material in the sample since the amount of material is reduced from the top of the column to the bottom.

Rows a–d are serial 5-fold dilutions of starting material. Letters over columns represent lettuce (L), chrysanthemum (M), tomato (To), tobacco (Tb), and thrips (Th). Thrips are small insects that transmit the virus between plants. Odd numbered columns are samples of material infected with tomato spotted wilt virus and even numbered columns are samples of the same type from uninfected tissue.

Nature of Sexual Reproduction in *Phytophthora* of Tropical Crops

Principal Investigator:
W. H. Ko, Dept. of Plant Pathology, UH

Nature of Project

Species of *Phytophthora* are important pathogens of numerous agricultural crops in the tropical and subtropical regions. Some species of *Phytophthora* are homothallic and are capable of completing their life cycle by single isolates. Other species are heterothallic, however, and require the presence of opposite mating types known as Al and A2 for the formation of the sexual stage of the life cycle. There was no logical explanation for such phenomena. Another problem encountered is that low and inconsistent frequency of oospore germination is the main obstacle in obtaining reliable data for genetic studies of *Phytophthora* This research has led to the discovery of a novel mode of sexual reproduction in *Phytophthora* and the development of an effective method for inducing oospore germination of *Phytophthora.*

Major Achievements

By using resistance to chloramphenicol and streptomycin as genetic markers, and by comparing the progenies from direct mating and mating on the opposite sides of the polycarbonate membrane, we found that all the progenies from the crosses between two different mating types of the same species are of uniparental origin. Our results show that heterothallic *Phytophthora* are actually homothallic but require sex hormones produced by the opposite mating type for induction of sexual reproduction, and that sexual reproduction of homothallic *Phytophthora* is dependent on hormones produced by themselves. This kind of hormonal heterothallism and homothallism in *Phytophthora* represents a novel mode of sexual reproduction in the biological world.

Importance to Users

The discovery of hormonal induction of sexual reproduction by single parents in *Phytophthora* suggests that in the distant future, it may be possible for scientists, through genetic engineering, to produce animals with reproductive systems that can be hormonally regulated at will. The new mode of sexual reproduction may provide new direction for breeding and production of livestock. It may also reveal new possibilities for aquaculture. For example, genetically engineered sturgeon and salmon may be able to produce offspring, all of which can be hormonally induced to lay eggs continually at certain time intervals for the caviar industry.

After trying numerous methods in preliminary tests, we found a very effective way of inducing oospore germination of *Phytophthora parasitica* (Figure 8). The method consists of exposing oospores to light during maturation after their formation, and treating oospores with 0.25 percent KMn04 for 20 min before incubation on agar medium containing basal salts, glucose and lecithin. Germination of oospores commenced within 24 hours under such conditions and was more than 90 percent after 10 days. The same method was also effective in inducing oospore germination of *Phytophthora infestans* and *Phytophthora palmivora*. In addition to genetics, this information should also be useful in studies of the biology and control of this group of pathogens.

Figure 8. Induced germination of a *Phytophthora parasitica* oospore.

Microbiological Control of Soil-Borne Pathogens in Tropical Soils

Principal Investigator:
W. H. Ko, Dept. of Plant Pathology, UH

Nature of Project

Suppressive soils are usually recognized by consistent observation of lower incidence of a plant disease in a certain area in contrast with that in the nearby area. The chances of encountering this phenomenon in the field are very small, and the number of this type of suppressive soils reported so far is relatively few. Project investigations have resulted in the development of a simple screening method for detecting pathogens-suppressive soils in nature and the discovery of plant disease control methods through exploitation of antagonistic mechanisms of natural suppressive soil.

Major Achievements

A simple method was developed for screening soils for pathogen suppression by comparing spore germination on surfaces of soil blocks placed on glass slides. By using the screening method described above, a Hawaii soil was found to be suppressive to *Pythium splendens* and cucumber damping-off caused by this pathogen (Figure 9). Experimental results revealed that a combination of calcium and high microbial population is responsible for the inhibition effect of the suppressive soil. In field trials, 86 percent control of cucumber damping-off was obtained by mixing soil in the small planting holes with lime to increase calcium content and alfalfa meal to increase microbial population.

In addition to inhibition of the pathogen, calcium increased the host resistance to the pathogen, and both calcium and alfalfa meal also stimulated plant growth. The advantages of this method are that it is inexpensive and non-hazardous. It has the potential of being used to control other plant diseases caused by *Pythium* spp.

Importance to Users

Since the development of this method, soils suppressive to the following pathogens have been detected: *Pythium splendens*, *Phytophthora capsici*, *Phytophthora cinnamomi*, *Fusarium oxysporum*, and *Rhizoctonia solani*. Through understanding nature's way of suppressing the pathogens, it may be possible to develop effective and non-hazardous means of controlling diseases caused by these pathogens.

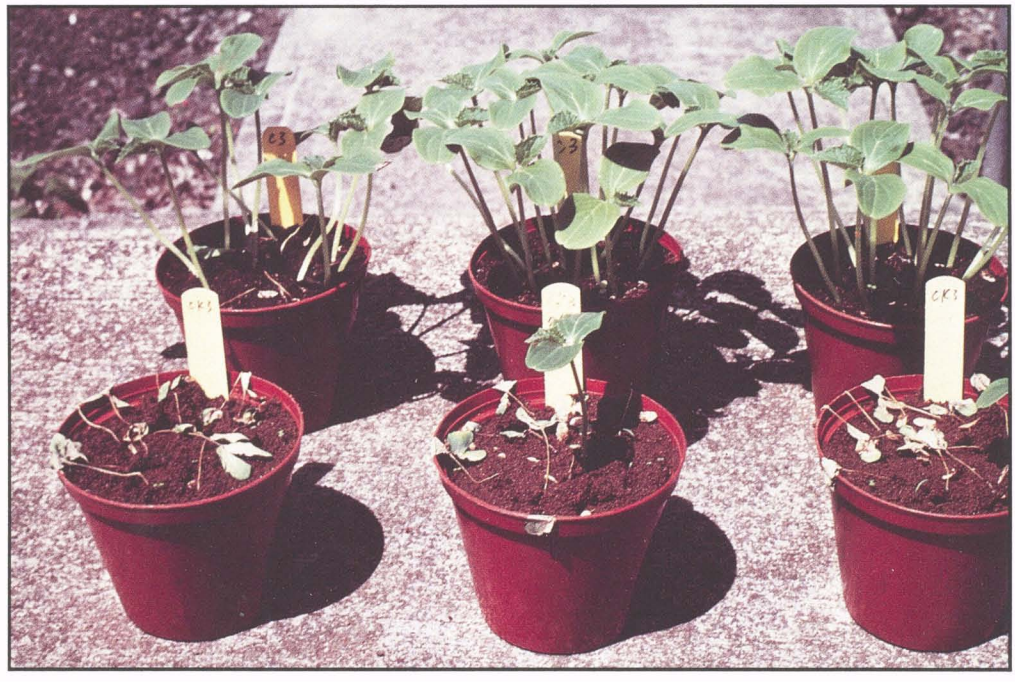


Figure 9. Damping-off of cucumber seedlings caused by *Pythium splendens* in conducive soil (front row) and suppressive soil (back row). Both soils were infested with the same amount of sporangia of *P. splendens*.

Breeding Papayas Resistant to Virus Diseases Through Interspecific Hybridization

Principal Investigator:
R. Manshardt, Dept. of Horticulture, UH

Nature of Project

The major objective of the project was to identify sources of resistance to papaya ringspot virus (PRV) among wild relatives of papaya and introduce genes for PRV resistance from the wild species into commercial papaya cultivars by interspecific hybridization.

Major Achievements

Eight *Carica* species were screened for resistance to PRV by a combination of methods, including rating of disease symptoms, transmission bioassays, and use of ELISA and a cDNA diagnostic probe. Four species were found to be resistant, and these were successfully hybridized with papaya cultivars by means of embryo rescue techniques. Two of the crosses were quite vigorous and have been tested for PRV resistance. *Carica papaya* x *C. quercifolia* F_1's have never shown PRV symptoms, even after repeated inoculation, while *Carica papaya* x *C. pubescens* F_1's have effective resistance under disease pressures normally existing in the field, but suffer an acute reaction if manually inoculated. In spite of formidable sterility, pistillate plants of these two F_1's were successfully backcrossed to papaya using embryo and ovule rescue techniques. Backcross progeny were all sesquidiploids, derived from unreduced eggs of the F_1's parents.

Importance to Users

Genetic resistance to PRV in commercial papaya cultivars is a possibility, if the sterility in the interspecific hybrids and derived sesquidiploids can be overcome. Further backcrossing to papaya is under way to return progenies to the diploid level and increase fertility.

Identification and Characterization of *Phytophthora* Blight Resistance in Papaya

Principal Investigators:
R. Manshardt, Dept. of Horticulture, UH
M. Aragaki, Dept. of Plant Pathology, UH

Nature of Project

The major objective of the project was to develop a greenhouse screening procedure that permits rating the development of *Phytophthora* blight symptoms in 'Kapoho' papaya seedlings over a one-week period. The inoculation procedure was then employed to screen different papaya genotypes for blight resistance relative to the 'Kapoho' standard.

A secondary objective was to determine the correlation of blight resistance and root rot resistance, both caused by *Phytophthora*, in different papaya genotypes.

Major Achievements

A procedure for screening papaya seedlings for resistance to *Phytophthora* blight was developed, using the cultivar 'Kapoho' as a standard. Cultivars identified by the screening procedure as having some resistance to *Phytophthora* blight were the same as cultivars previously identified as having resistance to *Phytophthora* root rot, suggesting a common mechanism of resistance.

These observations of resistance in 8-week-old seedlings by the screening procedure correlate well with the resistance levels observed in older trees under field conditions, and help establish the seedling screening procedure as a valid means of assessing *Phytophthora* resistance in papaya germplasm.

Importance to Users

Papaya lines which were observed to fare better than, or as well as, the standard cultivar 'Kapoho,' including 'Waimanalo,' line 40 and line 8, are being incorporated in a breeding program to increase *Phytophthora* resistance in papayas. Improved cultivars will eventually reduce the significant losses caused by root rot and blight in commercial fields throughout tropical regions.

Biological Analysis of *Thrips palmi* in the Pacific Basin

Principal Investigators:
R. F. L. Mau, Dept. of Entomology, UH
M. W. Johnson, Dept. of Entomology, UH
J. DeFrank, Dept. of Horticulture, UH
S. C. Welter, Dept. of Horticulture, UC-Berkeley

Nature of Project

Thrips palmi Karny (Thysanoptera: Thripidae) is an insecticide-resistant Indonesian species recently established in numerous areas in and surrounding the Pacific Basin. It has been found from Pakistan to the French West Indies. Little is known about this species, perhaps because it is not regarded as a pest in Indonesia. Due to the pest's wide host plant range, successful establishment in the continental United States would probably seriously impact fruit and vegetable production in the sun belt from California to Florida. Pesticide applications rarely suppress *Thrips palmi* populations and many times cause increases. The exact mechanism behind this positive response to pesticide use is not completely understood, but is believed to result from natural enemy destruction. Research is directed towards understanding the 1) influence of crop and weed hosts on *Thrips palmi* biology; 2) pest impact on the physiology, growth and productivity of common crop hosts; and 3) importance and potential of natural enemies in regulating *Thrips palmi* numbers.

Major Achievements

Appraising *Thrips palmi* injury can be difficult and time-consuming. A new technique was developed, the Welter leaf dye method, which significantly improves injury estimates. This technique requires immersion of injured leaves in a solution of basic fuchsin dye followed by a distilled water rinse. Leaf surfaces that have sustained thrips injury become dark red. Currently, the potential of estimating damage using a video/computer interface is being determined.

The impact of *Thrips palmi* foliage feeding on cucumber photosynthesis was estimated using a Li-Cor porometer. Laboratory studies showed highly significant correlations between percent leaf area damaged by *Thrips palmi* and changes in photosynthesis. Photosynthesis was reduced by 56 percent when 80 percent of the total leaf surface was damaged. These data have been referenced in the CRC *State of the Art Series* on plant-insect interactions. Field studies on cucumber have shown *Thrips palmi* to cause up to 80 percent reductions in normal photosynthesis rates. Similar impact on photosynthesis was produced by the western flower thrips, *Frankliniella occidentalis* (Pergande). These studies are the first to show effects of thrips feeding on photosynthesis. Reductions in photosynthesis may reduce plant growth and fruit production by destroying the plant's mechanism to produce much needed sugars.

Importance to Users

Identification of thrips species in a given crop is important with respect to predicting the need for control and selection of effective management tactics. Silver-colored plastic mulch beneath plants can significantly reduce *Thrips palmi* immigration into green peppers as compared to plant mulches (i.e., oats, ryegrass, rhodesgrass) or bare ground. Unfortunately, benefits of the silver mulch decrease as the plant canopy becomes fully established. Silver mulch did not influence *Frankliniella occidentalis* populations present in study plots. Cucumber may also be infested by mixed populations of *Thrips palmi* and *Frankliniella occidentalis*. Field studies show that cosmetic injury to fruit is predominantly caused by *Frankliniella occidentalis*. This may result from the thrips' habit of congregating in the cucumber flowers for mating. This species is also commonly found on the fruit and leaves. In contrast, *Thrips palmi* injury appears to be confined to the foliage, where it is commonly found. In the long run, *Thrips palmi* injury could reduce the overall weight and size of fruit as compared to the feeding scars on fruit produced by *Frankliniella occidentalis* that reduce the visual quality of the fruit, but not fruit size and weight.

Upon introduction to Hawaii, and probably most areas, *Thrips palmi* was not accompanied by those natural enemies associated with it in Indonesia where it evolved. This fact in itself could explain this species, sudden rise to pest status upon introduction into climatically suitable environments. Our studies show that thrips predators, present in Hawaii prior to *Thrips palmi* arrival, have broadened their prey range to include this pest. Most significant of these predators are the anthocorid *Orius insidiosus* (Say) and the predatory thrips *Franklinothrips vespiformis* (Crawford).

Elimination of these beneficial species by pesticide applications may increase *Thrips palmi* numbers. Recently, two parasitoids have been imported as possible biological control agents of *Thrips palmi*. These are the greenhouse thrips parasitoid *Thripobius semiluteus* (Boucek) and the onion thrips parasitoid *Ceranisus brui* (Vuillet), received from researchers in California and Japan, respectively. Studies are under way to determine if these species will parasitize *Thrips palmi*.

Work continues on the various areas described above. Introduction of *Thrips palmi* into the continental United States is a real possibility. If this occurs, these studies will provide important information with regard to management of this species.

Biological Control of the Weed *Chromolaena odorata* (Asteraceae)

Principal Investigators:
R. Muniappan, University of Guam
M. Marutani, University of Guam

Nature of Project

Chromolaena odorata is a neotropical weed introduced to Singapore in Asia in the mid 1800s and to Nigeria in 1937. Since then it has spread to most humid tropical regions in Asia and Africa. Currently it is a serious problem in the Philippines, Vietnam, Laos, Cambodia, Indonesia, Malaysia, Thailand, Burma, Bangladesh, Bhutan, India, Sri Lanka, Nigeria, Ghana, Ivory Coast, Cameroon, and South Africa.

C. odorata forms rambling bushy thickets. It is a problem in plantation crops, forests, game reserves, pastures, and vacant lands. It was noted to be a problem in the pastures of Rota in 1980 and became a problem on Guam, Tinian, and Saipan around 1983. As this weed is toxic and unpalatable to livestock, many pastures in Rota were abandoned due to its infestation. Because *C. odorata* occurs in marginal land in high density and over large areas, chemical and mechanical control methods are not feasible.

Major Achievements

In 1984, shipment of *Pareuchaetes pseudoinsulata* was received from the Commonwealth Institute of Biological Control, India, and a laboratory culture was established. In mid-1985, *P. pseudoinsulata* was field-established on Guam (Figure 10). Later, *P. pseudoinsulata* was introduced and established on Rota, Tinian, Saipan, and Aguijan. By the end of 1986 it had defoliated over 25,000 hectares of *C. odorata* in the Northern Mariana islands (Figure 11). Continuous defoliation has caused death of most bushes of *C. odorata. P. pseudoinsulata* has reduced the population of *C. odorata* in the Northern Mariana Islands, and the host and the natural enemy have attained a natural balance below the threshold level.

Importance to Users

In February–March 1988, an International Workshop on Biological Control of *C. odorata* was held in Bangkok, Thailand. As an outcome of this workshop, a newsletter and a proceedings were produced and distributed.

Currently, *P. pseudoinsulata* is being shipped to Thailand and Yap for biocontrol of *C. odorata*. South Africa and Pohnpei have indicated their interest in receiving *P. pseudoinsulata* in the near future. France is interested in taking up a *C. odorata* biocontrol project in Ivory Coast and Cameroon in West Africa. The Australian Centre for International Agricultural Research is planning a project in the Philippines.

Chromolaena odorata Newsletters #1, #2, and the Proceedings of the First International Workshop on Biological Control of *Chromolaena odorata* are available upon request from the Principal Investigators.

Figure 10. The *Pareuchaetes pseudoinsulata* larva, a natural enemy to *C. odorata*.

Figure 11. ***C. odorata*** **being defoliated by** ***P. pseudoinsulata*** **in Saipan.**

Biology and Biological Control of the Red Coconut Scale, *Furcaspis oceanica* (Lindinger)

Principal Investigators:
R. Muniappan, University of Guam
M. Marutani, University of Guam

Nature of Project

The red coconut scale, *Furcaspis oceanica*, is an endemic pest of coconut in the Caroline and Marshall Islands (Figure 12). During World War II, it was introduced to Saipan in the Northern Mariana Islands and became a serious pest of coconuts in 1948. An encyrtid parasite, *Adelencyrtus oceanicus*, was introduced to Saipan from Ulithi Atoll. *A. oceanicus* and another local parasite, *Rozanoviella* sp., have effectively suppressed the red coconut scale on Saipan.

In the early 1970s, the red coconut scale was introduced to Guam. Currently the scale is prevalent in the central part of Guam, but it has not been established in the southern and northern parts of Guam.

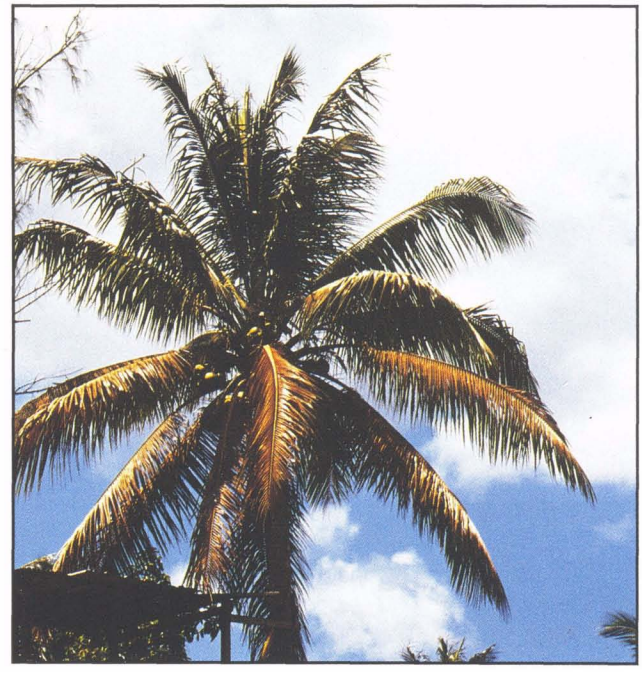


Figure 12. Coconut tree infested by coconut scale.

Major Achievements

Initial surveys proved the occurrence of three species of *Aphytes* and a *Rozanoviella* sp. on red coconut scale in Guam (Figure 13). In March–April 1988, the parasite *A. oceanicus* was collected in Ulithi Atoll and released on Guam. The establishment of this parasite on

Figure 13. Red coconut scale on leaf surface.

Guam is yet to be confirmed. Meanwhile, distribution of red coconut scale and its local parasites, dispersal of its crawlers, and preference for different parts of the coconut tree by the scale are being studied.

Importance to Users

Once the parasite *A. oceanicus* is established on Guam, it is expected to bring down the population of the *F. oceanica* to a very low level as happened in Saipan.

Cross Protection As a Means for Controlling Papaya Ringspot and *Citrus tristeza*

Principal Investigator:
R. Namba, Dept. of Entomology, UH

Nature of Project

Papaya ringspot virus (PRV) is a devastating disease of papaya wherever papaya is grown. Prevailing control methods are temporary or inadequate.

Cross protection, a phenomenon in which plants systemically infected with one strain of a virus are protected from the severe effects of a second strain of the same virus, was shown to be a promising method of control of PRV in a previous study of which this project is a continuation.

A basic requirement for cross protection is a satisfactorily mild protecting strain. Since no natural mild strain of PRV was found in the field, artificial mild strains had to be produced.

Major Achievements

A method was developed wherein sap from plants infected with the severe field strain was treated with nitrous acid, a powerful mutagenic chemical. The treated sap was then rubbed onto leaves of *Chenopodium quinoa*, a local lesion host of PRV. Each lesion was separately administered to papaya seedlings and in this manner the mild strains were isolated. This method proved to be reliable, and in recent years the technique has been used by scientists in Taiwan and Thailand to produce their own mild strains from their native field strains.

If cross protection is to be used extensively, an efficient, rapid method of mass inoculation of the mild protecting strain is required. A method was developed in which the papaya seedlings were infected with the mild strain of PRV by use of compressed-air spray guns. Extracted sap of mild strain infected plants was mixed with 600 mesh carborundum and sprayed onto the leaves of the seedlings with pressures of 4–6 kg/cm^2 at a distance of 10 cm or 8 kg/cm^2 at 20 cm. One person

could inoculate 10,000 seedlings in 2 hours with 100 percent infection with this method.

Importance to Users

Extensive field trials in Taiwan and to a lesser degree in Hawaii have shown cross protection to be a viable method. In Taiwan, protected trees produced many more fruits than unprotected trees and, more importantly, produced 100 percent or greater income for the farmers (Figures 14 and 15). Last season a million protected trees were planted. The production of protected trees has now been transferred to government-monitored private nurseries.

In Northeast Thailand, where papaya is a valuable green salad staple, 30,000 protected papaya plants were distributed to 20 villages for backyard planting. In a recent trial in Hawaii, Line 8 cultivar of papaya plants protected with mild strain HA 5-1 were grown successfully in the Waianae District where in recent years PRV has curtailed the growing of papaya.

Cross protection field trials are also being conducted in Homestead, Florida; Veracruz, Mexico; and Jordan Valley, Israel.

Cross protection has been used extensively and successfully for citrus tristeza virus (CTV) in Israel and Brazil. It has not been a widespread practice in the USA. Many mild protecting strains are available in nature and there is a need for evaluation under various conditions to determine which ones would be best.

Hawaii is an excellent site for CTV study because it has many very severe strains and all of the known vectors of CTV. Various citrus varieties, to be used for rootstocks, were tested under nursery conditions to determine susceptibility and rate of dissemination of CTV. Within 6 months, even under moderate CTV and aphid challenge pressures, 85–100 percent of the rough lemon, *C. macrophylla* and Volkamer became infected. Cleopatra mandarin and Carrizo citrange showed excellent resistance.

Pink Shadduck pummelo was found to be highly resistant to CTV even under very severe virus/vector pressures. Hybridization studies show that resistance is an inheritable characteristic. It will be useful as a breeding parent for creating new CTV-resistant varieties of citrus.

Eleven Hawaii CTV isolates have been established at Beltsville, Maryland, as part of a worldwide collection. They are involved in such studies as host range, rootstock selection, and strain discrimination by use of monoclonal antibodies, and used as challenge isolates for mild protecting strains from other parts of the world.

Transmission studies with *Aphis gossypii* show that there are differences in rates among the various CTV isolates. Very high rates were obtained with isolates from Israel, Japan, Brazil, and Spain, which means that these exotic isolates can become readily disseminated if accidentally introduced.

Figure 14. Unprotected papaya trees with severe papaya ringspot symptoms and very little fruit protection (Taiwan).

Figure 15. Papaya trees protected with mild strain of PRV with resulting heavy fruit production (Taiwan).

Management of *Ostrinia furnacalis* on Corn in Micronesia

Principal Investigators:
D. M. Nafus, University of Guam
I. H. Schreiner, University of Guam

Nature of Project

In Asia and some of Micronesia the Asian corn borer is a severe pest of corn. On Guam, very high numbers of corn borers are present throughout the year and frequently corn plantings are totally destroyed. Currently, sweet corn and corn for animal feed or making tortillas are imported from the U.S., but both could be produced locally to meet market needs if this pest problem could be resolved. This project was initiated to seek improved ways to control the corn borer.

Major Achievements

A combination of a cultural control (detasselling) and an insecticide was shown to be more effective in controlling the corn borer than current procedures. Insecticides alone occasionally fail to control corn borers, resulting in high yield losses even with twice-weekly insecticide use. In particular, we found this happens because corn borer larvae move into parts of the plant which are difficult to penetrate with insecticides. For example, young larvae were found between the husks of the ear and inside the florets in the tassels; these are areas which are almost impossible to reach without systemic insecticides.

By treating the plants with the bacterial insecticide *Bacillus thuringiensis* (B.T.) and by removing the corn tassels, corn borer populations were reduced as much as 95 percent. By themselves, insecticides reduced corn borer populations a maximum of 60 percent.

Over 200 field corn inbreds and 52 commercial varieties or breeding lines of sweet corn were screened for resistance to the Asian corn borer *Ostrinia furnacalis*. Resistance to leaf-feeding was found in several inbreds. Resistance to tassel-feeding was found for the first time. The 10 varieties with the most resistant leaf rating scores were A619, Hi40, H60, AntC5-S5, HIX4231, CIM.T 11ES, C166, INV138, Mp496, and Hi31. The 10 varieties with the most resistant tassel ratings were ICAL29, ICAL25, MP68:616, Hi34, HIX4267, Fla 2BT 106, PhilDMR6-S5, HI41, H95, and NC246. There was a significant relationship between leaf ratings and tassel ratings.

Over 9000 eggs, 1100 larvae, and 3600 pupae were examined for the presence of parasitoids. Four parasitoids were found which parasitized about 20 percent of the eggs

and less than 1 percent of the pupae. *Nosema* sp. was found on Guam parasitizing borer larvae. Less than 10 percent of the larvae were infested.

Several alternate hosts were identified and the growth and survival of the borer was determined on some of these hosts. Some of the more important alternate hosts were introduced as forage grasses.

Importance to Users

Host plant resistance is an inexpensive and easy way to control pest problems. The potential for breeding resistance to the Asian corn borer is good. Corn inbreds which can serve as initial breeding stock to develop resistant varieties have been identified.

If farmers combine tassel removal with B.T. sprayed once weekly, substantial reductions in borer population and consequent improvements in yields can be achieved. Detasselling requires about 21 person-hours per hectare, but can increase yields by 20 percent and provide a net return to Guam farmers by $1000–2000 per hectare.

Farmers are receiving little help from biological control agents. The importation of new natural enemies is needed to reduce populations of the corn borer on Guam.

Farmers with plenty of alternate hosts should consider management of these plants around their fields. Planting should be done after these hosts have dried up and corn borer populations are low. New forage grasses should be screened for their potential as corn borer hosts, as the introduction of these grasses is implicated in increasing the severity of the corn borer problem on Guam.

Biology and Control of Mango Shoot Moth and Mango Blotch Miner on Guam

Principal Investigators:
I. H. Schreiner, University of Guam
D. M. Nafus, University of Guam

Nature of Project

Studies of mango on Guam had shown that over 50 percent of the foliage of mangoes on Guam was being consumed by caterpillars of *Penicillaria jocosatrix.* These caterpillars also had a significant impact on flowers, causing both direct damage and reducing the ability of the trees to produce flowers. Leaves are further damaged by a cecydomyiid blotch miner, which was not taxonomically identified at the beginning of the project. This project was directed at studying the biology of *Penicillaria* and developing methods to control this pest. In addition. we wished to determine whether the blotch miner was a significant pest.

Significant Achievements

Studies of the biology of the caterpillars showed that they prefer young leaves, and that survival of young larvae decreases significantly as the leaves begin to mature. The larvae also feed very successfully on flowers. In one study, yield of fruit increased 50 percent when caterpillars were controlled with insecticides. Three biological control organisms from India and a *Trichogramma* egg parasite from California were imported and released. Two of the larval parasites from India became established in late 1986 and are now parasitizing up to 40 percent of the larvae. Recently, eggs have also been found to be parasitized, but we have not yet obtained confirmation as to whether the *Trichogramma* also is established. Long-term monitoring has shown that the caterpillar population has been substantially reduced since July 1987, compared to previous levels.

Specimens of larvae and adults of the mango blotch miner were determined to be an unidentified species of *Procontarinia*, which is now being described. Although this insect has only been reared in Guam, damage which appears to be caused by this insect also occurs in the Northern Mariana Islands, and in the Western Carolines. The blotch miner attacks only very young leaves about 2–4 cm long. Long-term monitoring showed that blotch miner populations are generally low, with only occasional outbreaks. These outbreaks are loosely correlated with periods of very rainy weather.

Importance to Users

Substantial control of the *Penicillaria* due to the biological control agents occurred. Trees have more leaves and in 1988, fruit yields of monitored trees were 20 times higher than they were in 1986 and 80 times greater than they were in 1987. Although additional data will be needed to determine whether this is a long-term change due to the biological control agents, it seems likely that most homeowners will no longer have to spray to get a reasonable yield from their trees. *Procontarinia* has been identified, and determined to be an unimportant pest most of the time, although further assessment of its outbreak potential and its relationship to anthracnose needs to be made.

Computer-Aided Management of Insecticide Resistance in Tropical Pests

Principal Investigators:
B. Tabashnik, Dept. of Entomology, UH
M. Johnson, Dept. of Entomology, UH

Nature of Project

Pest resistance to insecticides threatens agriculture and human health throughout the world. Resistance to insecticides has been documented for more than 440 species of insects and mites, with costs due to resistance estimated at greater than $130 million annually in the U.S. alone. Resistance management aims to prevent or delay development of resistance to insecticides in pests. Our project is developing and testing computer-based simulation models to understand and predict the onset of resistance development. Our experimental work has focused on the diamondback moth, a global pest of cabbage and related vegetables. To coordinate efforts to combat this pest we have joined with researchers in New York, California, Texas, Florida, Georgia, Ohio, Wisconsin, Indiana, Washington, Oklahoma, Connecticut, and Minnesota to initiate a national diamondback moth research program.

Major Achievements

We first devised an efficient and reliable technique for monitoring insecticide resistance in the diamondback moth (Figure 16). A detailed comparison showed that our method has several advantages over the technique previously recommended by the Food and Agriculture Organization (FAO) of the United Nations. Our method has been published by the FAO and adopted as the standard technique for the U.S. national program. Because monitoring of resistance is an essential component for management, our technique could greatly aid efforts to delay or prevent resistance.

Importance to Users

The project has developed a computer model of pesticide resistance that provides a safe, fast, and inexpensive way to project the potential short- and long-term consequences of various management options (Figure 17). Predictions from the model agree with historical data on resistance from Taiwan, Malaysia, Thailand, and Japan. Rigorous field tests of the model are continuing in Hawaii, but findings from our modeling approach have already been applied to resistance problems in other vegetable pests, as well as major pests of cotton, apple, and livestock in the United States.

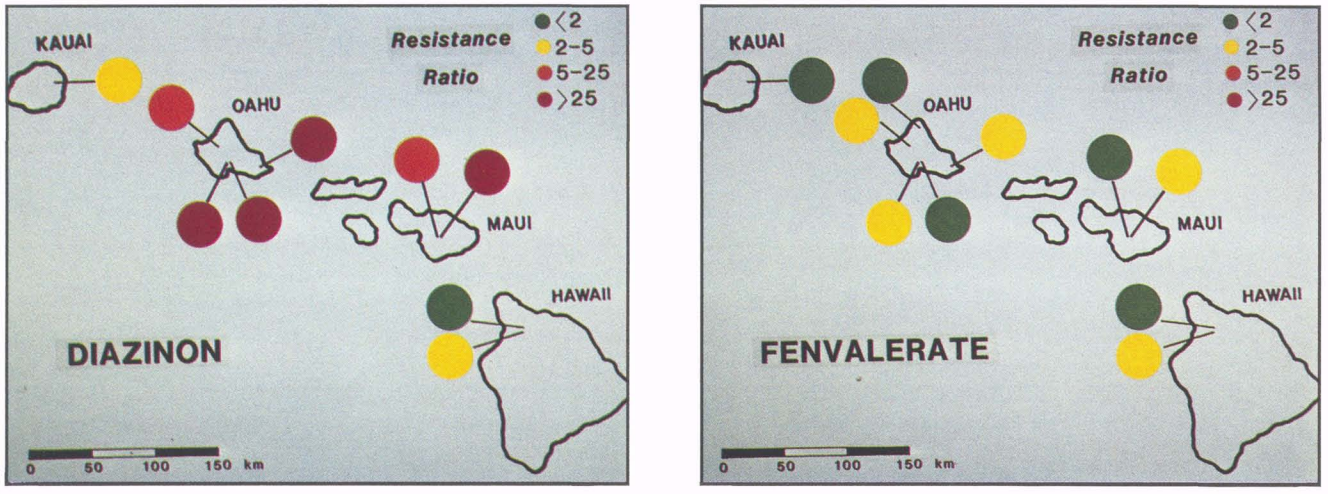


Figure 16. Diamondback moth resistance to insecticides in Hawaii. Resistance ratios indicate the resistance of field populations relative to a laboratory strain. Ratios were measured by determining the median lethal concentration (LC_{50}) for laboratory and field populations.

Left: **Diazinon. Several populations have high levels (>25-fold) of resistance to diazinon, which has been used for many years against the diamondback moth.**

Right: **Fenvalerate. Only low levels of resistance to this recently introduced pyrethroid were detected.**

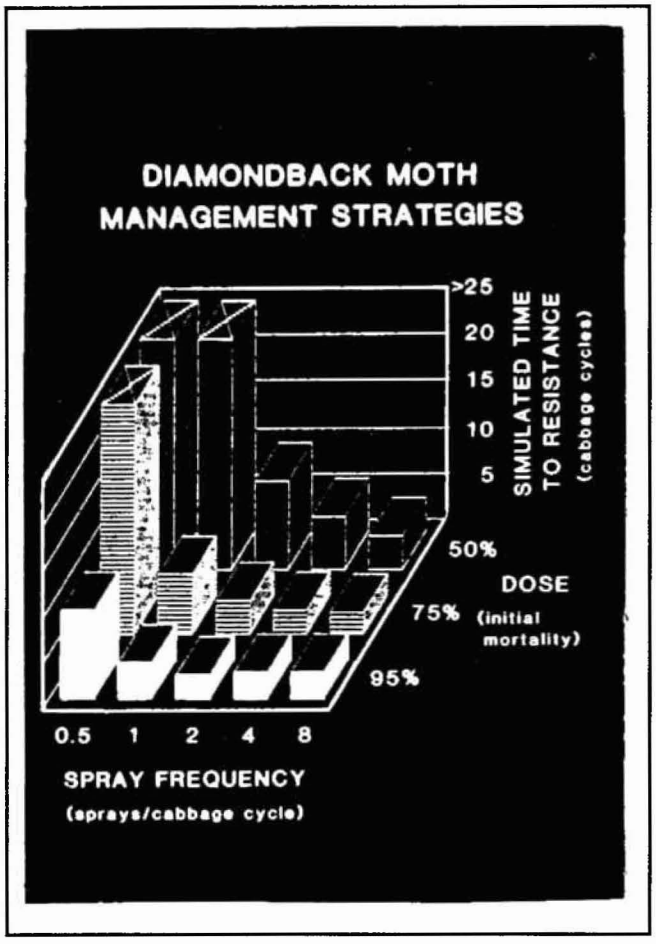


Figure 17. Effects of spray frequency and dose on simulated time for the diamondback moth to develop resistance to fenvalerate. Simulations show that reductions in spray frequency, dose, or both are needed to slow resistance development.

Rainfall Erosion Parameters and Impacts on Tropical Soil Productivity

Principal Investigator:
S. A. El-Swaify, Dept. of Agronomy & Soil Science, UH

Nature of Project

The project aims at quantifying the climatic soil-based, topographic, and management causes of soil erosion by water, the consequences of soil loss to productivity, and the effective means of rehabilitating eroded, highly-weathered, and nutrient-depleted tropical soils. Research has been conducted at three ecologically distinct locations representing moist (udic), semi-arid (ustic), and arid (aridic) regimes in Hawaii.

Major Achievements

A. Inherent soil loss parameters: Experimental values of erodibility were obtained for major Hawaii soils for the first time; predictive equations were also developed for estimating the erodibility of unknown soils. Rainfall erosivity was quantified, also for the first time, and iso-erodent maps were constructed for the islands using long-term historic precipitation records. These findings are among the earliest in the tropics. They presently form the bases for USDA Soil Conservation Service erosion prediction, and control recommendations in Hawaii, and they are in considerable demand for application in the Pacific and other tropical regions outside Hawaii.

B. Management parameters for conservation planning: Runoff and soil loss from pineapple watersheds generally exceeded those from sugarcane; neither values were considered "intolerable," however. The conservation of major cropping systems was significantly enhanced by use of legume intercrops; the likelihood of farmer adoption and assurance of sustainability can be increased if the intercrop carries the added advantage of providing a cash income, e.g., groundnut. Our work on this subject was invited and published as the leading article in the first volume of *Soil Technology*.

C. Erosion impacts on soil productivity: Extensive studies using simulated erosion on Oxisols revealed drastic changes in explorable rooting depth and the nutritional, physical, and biological quality of highly weathered soils for supporting crop growth. Severe depression resulted in the growth and yields of major crops (field corn, sweet corn, Irish potato, soybeans, sweet potato). The severity of the effect was different for the different crops, however. Full recovery of productivity was not possible with "restorative" fertilization alone. The potential added benefits of primary tillage were, however, difficult to detect in the presence of supplementary irrigation. Erosion impacts under strictly rainfed conditions are more severe and short-term. For these reasons and because of the limited resources of "typical" tropical small farmers, we have concluded that the "loss-tolerances" for highly weathered soils of the tropics are lower than those for their temperate counterparts. This work was partly published and a summary was included in CSRS's list of National Examples of Research Accomplishments for 1987.

Importance to Users

A. Soil Conservation Service, USDA: In the absence of an ARS/USDA team to meet the research needs of SCS in Hawaii, this project has provided several key inputs that are critical to SCS's erosion control activities in the state:

1) Predicting soil loss: The project now allows SCS to estimate soil losses and formulate effective conservation practices for agricultural lands in Hawaii, using more reliable soil erodibility and rainfall erosivity values than ever before. Up-to-date isoerodent maps and monthly erosivity distributions in use by SCS are direct products of research in the project; soil erodibility values, too, are determined with reference to our benchmark values. We continue to work closely with SCS to assure that they have access to the most recent information for implementing such compliance provisions as in the current Food Security Act (FSA). Concerns with real or perceived non-point pollution, particularly from macadamia orchards, has led us to work closely with SCS, Soil and Water Conservation districts, and the State of Hawaii Department of Health to formulate plans for quantifying the problem and suggesting solutions where needed.

2) Setting soil loss tolerances: A critical policy decision and management target in conservation planning is the setting of "acceptable" or "tolerable" soil loss, i.e., the rate of erosion above which soil productivity cannot be sustained economically and indefinitely. Our findings on erosion-productivity impacts indicate the high fragility of tropical soils. This would translate into lower soil loss tolerances than for temperate soils.

3) Low-input conservation and sustainability planning: Our research on the effectiveness of vegetal methods (such as legume intercropping) for erosion control offers an important alternative or at least a complement to the common reliance on structural measures. Not only is agronomic management affordable and inducive to high productivity, it is also a critical ingredient to careful "land husbandry" and, therefore, the long-term sustainability of agricultural enterprises.

B. Scientists, policy makers, and practitioners in the tropics: The need for quantitative data such as discussed above is so great, and the availability of it in the tropics is so scarce, that our information and simplified assessment methods have been in continuous demand for publication and/or presentation. Symbolic is the chapter "Soil Erosion by Water," in the *Handbook on Natural Systems for Development: What Planners Need to Know* (MacMillan & Co., 1983), the now out-of-print *Soil Erosion by Water in the Tropics* (HITAHR-UH Press, 1982), and the need to reprint our edited book *Soil Erosion and Conservation* (Soil Conservation Society of America, 1985). We have been requested to contribute to every major international conference, symposium, and workshop dealing with conservation in recent years. From 1987 to 1989 these have included the Workshop on Soil and Water Conservation (Kuala Lumpur), 5th Inter- national Soil Conservation Conference (International Soil Conservation Organization, ISCO, Bangkok), International Conference on Dryland Farming (USDA/AID, Bushland), First International Workshop on Soil Management and Small Holders Development in the Pacific Islands (IBSRAM, Honiara), International Conference on Sustainable Agriculture (Ohio State University, Columbus), International Workshop on Conservation Farming on Hillslopes (Chinese Soil Conservation Society, Taichung), and the 6th International Conference on Soil Conservation (ISCO, Addis Ababa). We have also designed short-term soil and water conservation training at the request of the USAID Mission in Indonesia and the Instituto de Pesquisas Technologicas, Brasil. Our membership has also been requested in a new committee for establishing a sustainability strategy for the Consultative Group on International Agricultural Research (CGIAR) and another established by the International Union for the Conservation of Nature and Natural Resources, Commission on Ecology, to conduct a global assessment of soil erosion.

Rhizobium-Soil Erosion Interaction in Highly Weathered Tropical Soils

Principal Investigators:
M. Habte, Dept. of Agronomy & Soil Science, UH
S. A. El-Swaify, Dept. of Agronomy & Soil Science, UH

Nature of Project

The loss of productivity following erosional losses of soil, by and large, has been attributed to adverse changes in chemical and physical properties. Since plant growth is significantly affected by soil microorganisms, a better understanding of the impacts of erosion on soil productivity cannot be achieved unless its impacts on microbial populations are also clearly understood.

The overall objective of our investigation was to evaluate the impacts of erosional soil losses on the abundance and activity of indigenous as well as introduced populations of rhizobia along with vesicular-arbuscular mycorrhizal fungi. Our ultimate goal was to manipulate these microorganisms for a cost-effective rehabilitation of highly weathered eroded soils of the tropics.

Major Achievements

This research has demonstrated that populations of indigenous rhizobia and vesicular-arbuscular mycorrhizal (VAM) fungi are adversely influenced by surface soil removal in excess of 7.5 cm (VAM fungi) (Figure 18) or 15 cm (rhizobia). Propagules lost during erosional soil losses must, therefore, be replaced before legumes grown in highly weathered eroded tropical soils could appreciably benefit from symbiosis with rhizobia and/or VAM fungi.

Studies have conclusively shown that the adverse influence of surface soil loss on rhizobial populations introduced in the Wahiawa Oxisol is strain specific, the poor survival of sensitive strains being explainable in terms of the loss of Ca. The adverse influence of erosional soil losses in this soil could thus be overcome either by increasing the supply of calcium in the soil or by selecting rhizobia that are insensitive to erosional soil losses.

We have shown that the losses of rhizobia and VAM fungi accompanying soil losses could be offset by a reinfestation of the eroded soils with the microorganisms. The symbiotic activities of the organisms are not restored, however, unless the soils are also compensated for lost nutrients. We have defined the levels of nutrients that are compatible with effective rhizobia and VAM symbiotic functions. These levels are substantially lower than what would be required to restore the productivity of the eroded soils in the absence of the microorganisms.

Our findings have revealed that the tolerance limits to erosion of rhizobial and VAM populations are greater than those of host plants or host-endophyte associations. Hence, it is possible to reduce further the need for purchased restorative inputs through a careful host genotype screening for tolerance to erosion.

Our investigation has also led to the development of nondestructive techniques for monitoring the development of the VAM symbiosis as a function of time. These techniques have revolutionized our understanding of the symbiosis.

Most of our findings are either published or are in the process of being prepared for publication. A total of 11 articles have been accepted for publication so far; seven are in refereed journals.

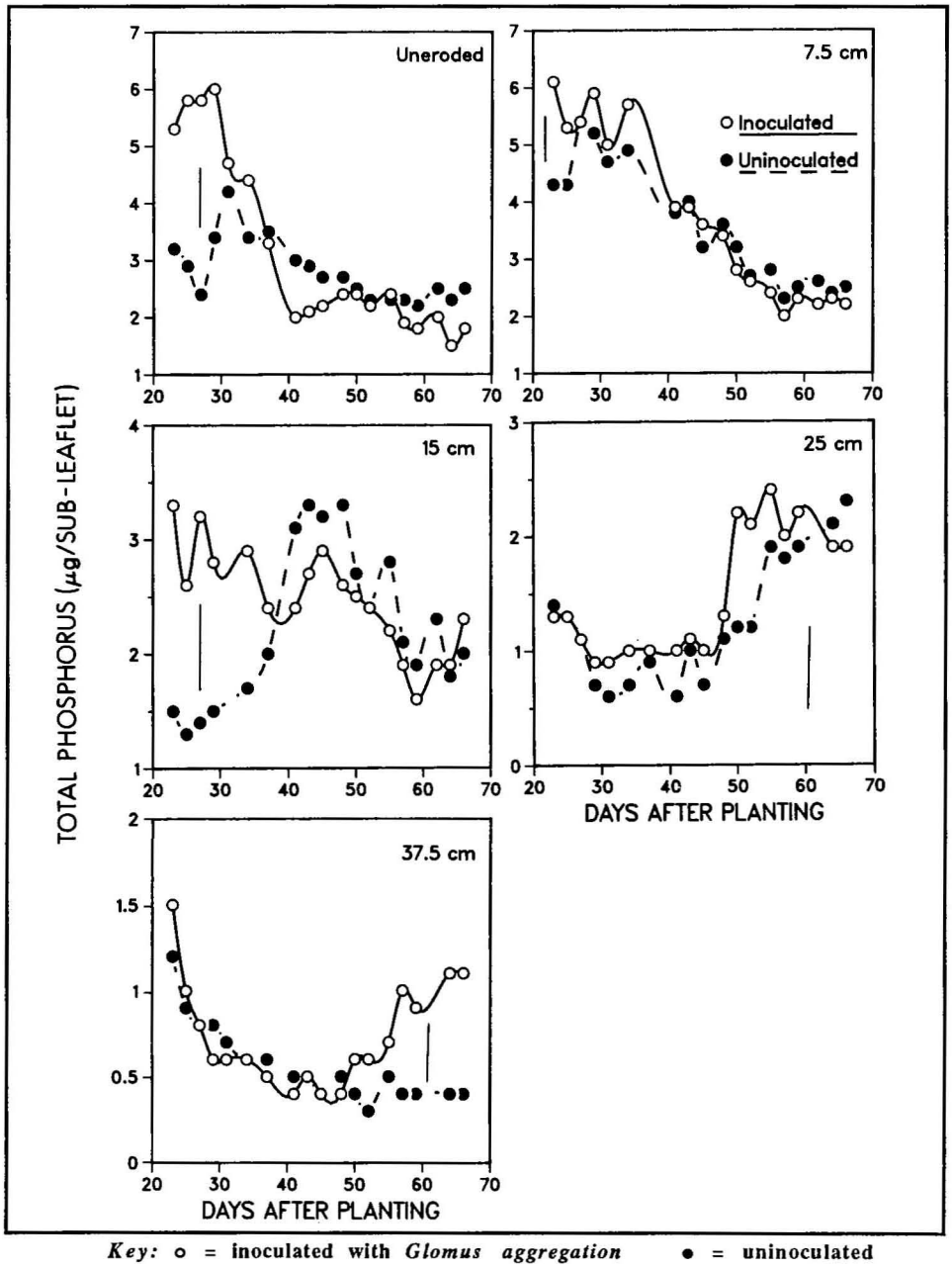
Importance to Users

This work represents the first strong evidence in support of a biological approach to the rehabilitation of eroded and degraded lands. It should be of utmost interest to conservation planners and workers because it offers a cost-effective alternative to the cost- and technologically-intensive approaches currently employed in the rehabilitation of eroded soils.

In the process of defining conditions required for a successful establishment of nodulated and mycorrhizal legumes in eroded soils, we have been able to determine the conditions that are critical for optimal functioning of the tripartite symbiosis between rhizobia, legumes, and VAM fungi. This information will contribute significantly toward the development of sustainable agricultural systems in the tropics.

The work will create awareness on the impacts of erosion on biological properties of the soil. The new ground broken by the investigation as well as the questions raised in the course of the investigation will stimulate researchers to launch more investigations in the same or related areas.

It is anticipated that environmentally-oriented users of the information and those concerned with conservation work in the developing regions of the tropics will give it their urgent attention. The methodologies developed will significantly impact VAM researchers.



Key: o = inoculated with *Glomus aggregation* ● = uninoculated

Figure 18. The influence of simulated erosion (surface soil removal) on VA mycorrhizal activity expresed in terms of P status of *Leucaena leucocephala* subleaflets.

A Correlation of Phosphorus and Sulfur Utilization Efficiencies with Specific Mineral Properties of Tropical Soils

Principal Investigators:
R. C. Jones, Dept. of Agronomy & Soil Science, UH
R. L. Fox, Dept. of Agronomy and Soil Science, UH

Nature of Project

Based on preliminary work with x-ray diffraction (XRD) line profile analysis, the prime objective was to test the effectiveness of XRD profile refinement and peak decomposition in predicting chemical and physical properties of soils. Of particular interest was to determine if XRD parameters that were previously unavailable could be extracted from digitized diffractograms and used to *quantify* those mineral properties that are most responsible for observed chemical reactions in soils. Before this project was initiated, soil minerals were *qualitatively* determined by identifying a series of peaks on a recorded strip chart. Now, by computer collection of digitized XRD intensities, there is the capability to extract information such as the area under unresolved, overlapping peaks (a measure of the *amount* of each mineral present), the exact peak positions (an indication of the extent of ionic substitutions in the crystal lattice), peak widths (values that are used to calculate the mean crystallite size of each mineral), and peak shapes that indicate the extent of crystal lattice defects (degree of crystallization). From previous studies it was clear that a particular soil chemical reaction could not be explained only on the basis of the minerals that were present, or even on the basis of how much of a given mineral was present. Phosphate sorption is an example of a chemical process that could not be explained solely on the basis of mineralogy alone. This project, therefore, addressed specific mineral properties such as surface area (amount present divided by the mean crystallite size), crystallite morphology (surfaces most likely to react with a given substance—phosphate or sulfate), and degree of crystallinity (the possibility of precipitation or mechanical entrapment).

Major Achievements

Two computer programs were written, one to collect digitized XRD intensities from a modified diffractometer and the other to profile refine and decompose the peaks. The program was given a unique name of *Pi'o Pili Pa'a*, which is Hawaiian for *curve close fit*, to distinguish it from earlier deconvolution/decomposition programs. It was used to refine and decompose the diffractograms of a number of different soil types having different chemical properties. The result was that the aluminum minerals were found to have low surface areas in the bauxite soils and did not correlate with phosphate sorption. Goethite, on the other hand, was found to account for at least 87 percent of the phosphate fixation in bauxitic soils. The results show that there is more phosphate sorption in the subsoil than at the surface although the concentration of goethite is higher at the surface. Through profile refinement and peak decomposition, it was found that because goethite had a much smaller crystallite size in the bauxite subsoil, the surface area was higher, even though there was a lesser amount of goethite present than at the soil surface. The implications of such a finding point to the potential loss of soil productivity in the case of the bauxite soils if the topsoil is removed either by excavation or erosion.

In surveying a number of other soils having similar pH values, mean crystallite size coupled with mineral abundance correlated well with phosphate sorption. The study brought to focus that in many cases there are multiple mineral-phosphate reactions. There are many cases where a single mineral may not correlate well with phosphate sorption; when several minerals are collectively considered, however, very high correlation coefficients are obtained. This study also reinforced the supposition that pH and organic matter content play an important role in phosphate sorption.

Importance to Users

The users of the techniques developed under this project will be soil scientists. For the first time XRD can be used to *quantify* the results of a mineralogical analysis. Quantification can now include previously unobtainable values as surface area of a single phase in a complex mixture such as a soil. By the use of curve fitting and peak decomposition, mineralogical analyses will have greater relevance than before in terms of predicting soil chemical behavior. Selected examples of the types of information collected by use of computer program *Pi'o Pili Pa'a* have been reported and displayed at four national and one international meetings. The papers presented have generated considerable interest, and requests for copies of the program are steadily being received. The program is being readied for free distribution including a booklet on its theory and use. The two requirements for the use of curve fitting and peak decomposition that may limit the number of soil scientists who can take advantage of the program are that x-ray diffraction intensities must be digitally collected and a main-frame computer should be used. Because of the program's size and the number of calculations that must be performed, small microcomputers would require as much as an overnight session to curve-fit one diffractogram.

Deficit Irrigation for Tropical Vegetable Crops

Principal Investigators:
I-P. Wu, Dept. of Agricultural Engineering, UH
T. Hori. Maui Extension, UH
B. Kratky, Beaumont Research Center, UH

Nature of Project

The project takes into account the non-uniform characteristics of irrigation systems and makes better use of water resources by allowing some portions of crop areas in the field to be under a water deficit situation. This concept can increase the irrigation application efficiency. A certain amount of crop yield reduction will result from deficit applications, however. The combination of water saving and resulting crop reduction poses an optimization problem which determines the optimal irrigation scheduling for the maximum return. This project involves theoretical development, computer simulation, and field experiments to address this problem.

Major Achievements

Two major achievements resulted from the irrigation experiments; one is the optimal evapotranspiration (ET) for Hawaii vegetables, and the other is a simple crop response model. The optimal ET for Hawaii vegetables is around 0.1 inch per day, and the crop response model can be expressed by a sloped straight line showing the increase of yield with the increase of ET and by a horizontal line at the peak yield showing no yield changes after the optimal point.

The theoretical analysis showed that the sloped portion of the crop response can be plotted dimensionlessly as a straight line expressing the effect on yield by deficit irrigation. The slope of the straight line, depending on the crop, is the reduction coefficient. Through increased understanding of the uniformity of a drip irrigation system using computer simulation, an optimal irrigation program is developed that can be used to determine optimal irrigation scheduling considering the uniformity of the irrigation system, crop reduction coefficient, cost of water, and the price of the crop yield.

Importance to Users

The immediate application is the new concept of optimal ET, which will provide a guide to farmers to determine the right amount of water to apply. The optimization program will help farmers maximize return during limited water situations by applying the concept of deficit irrigation.

A Potential Management Alternative for Highly-Diluted Swine Wastewater in the Tropics

Principal Investigator:
P. Y. Yang, Dept. of Agricultural Engineering, UH

Nature of Project

Two treatment alternatives, namely Horizontally Baffled Anaerobic Reactor (HBAR) and Intermittently Aerated Bio-Carousel Reactor (IABR) for diluted swine wastewater have been evaluated technically and demonstrated. Necessary design and operational criteria have also been developed. The capital and operational costs per pig for these two processes are about the same.

For real tropical application, the capital cost in a heating device for HBAR can be saved and the cost per pig basis for HBAR can be reduced to 80 percent of the original estimated cost. Some advantages that the IABR has over the HBAR, however, are the ability to remove nitrogen much more effectively, greater stability, and faster start-up period. To select a treatment alternative for highly diluted swine wastewater in the tropics, basic requirements need to be considered such as land availability and cost, energy utilization, water consumption, fertilizer/soil conditioner utilization, requirements of treated effluent discharge standards, manageability, and reliability.

Major Achievements

A full-scale unit incorporating the IABR process, having a volume of 76 m^3, has been constructed on the island of Kauai (Figure 19). This unit was used to treat the diluted supernatant of settled pig wastewater generated from a 2000-head swine farm. The settled or concentrated swine manure was treated by an anaerobic digestion unit. Also, a similar IABR unit (40 m^3) has been planned to treat the diluted supernatant of settled pig wastewater on the island of Oahu. The concentrated or settled manure will be also treated by an anaerobic reactor.

Importance to Users

The separated treatment of concentrated and diluted swine wastewater can provide an effective treatment and a

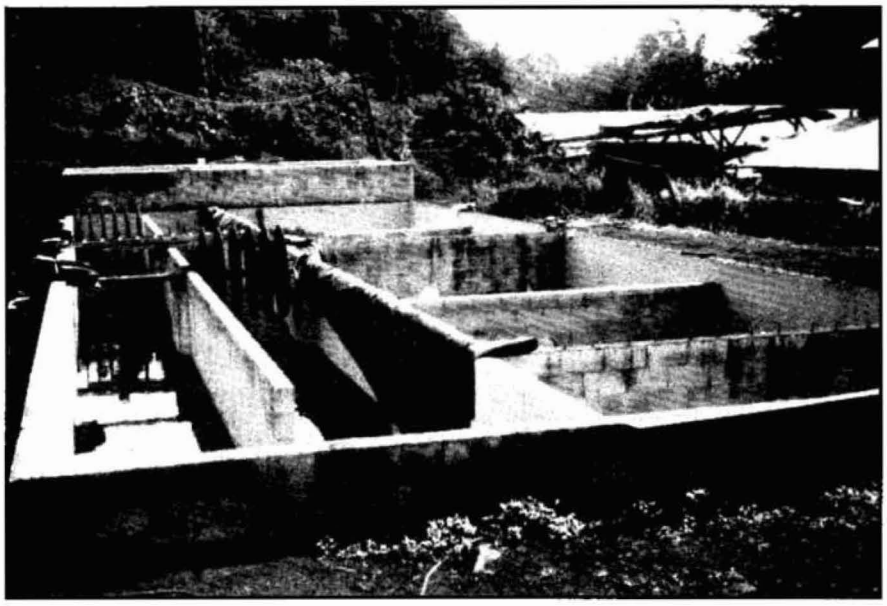


Figure 19. Full scale intermittently aerated bio-carousel reactor.

reduction of land space requirements. The development of these two treatment alternatives for the diluted swine wastewater can provide the following impacts and benefits for the swine production system in the tropics:

- Improvement of the farm community environment and enhancement of the concept so diversified agriculture can be achieved.
- Rural water supply systems can be protected and high incidence of some tropical diseases can be eliminated.
- Wastewater effluent discharge standard can be established in the tropics.
- Integrating swine wastewater treatment alternatives with crop production and aquaculture production can be properly developed and can increase both crop and aquaculture production in the tropics.

Improved Culture Techniques for the Chinese Catfish (*Clarias fuscus*) in Hawaii

Principal Investigators:
A. Fast, Hawaii Institute of Marine Biology, UH
M. Young, Hawaii Institute of Marine Biology, UH

Nature of Project

The clariid catfish *Clarias fuscus* was introduced in Hawaii more than 90 years ago by immigrants from its native Taiwan. Although the fish has since become well established in freshwater streams and ponds on most of the Hawaiian islands, it has never been abundant. Nevertheless, it has remained a favorite food fish for Chinese, Filipinos, and other Asian ethnic groups who are familiar with clariid catfish. The problem has been the limited supply of wild caught fish and the lack of good culture techniques specific to this species in Hawaii.

Major Achievements

As a result of this project, we developed improved culture techniques and we have extended those results to the private sector.

Specific technical accomplishments of this research include the following:

(a) Determination of the natural seasonal spawning cycle: Under ambient conditions, *C. fuscus* will only mature and be capable of spawning for about 5 months each year, from May through September. Natural (non-induced) spawning is infrequent and may only occur for 2 or 3 months. The fish will not spawn in captivity without hormonal inducement of ovulation in the female to obtain eggs and sacrifice of the male to obtain sperm.

(b) Determination of maturation stimuli and development of induced maturation procedures: Through experiments with photoperiod and temperature treatments, we determined that temperature is the primary factor affecting ovarian development and maturation. Through this knowledge, we were able to develop practical techniques for egg production any month of the year.

(c) Induced spawning: We determined optimum hormone dose rates and dose timing to induce ovulation and production of viable eggs. We also developed methods of assessing "ripeness" in females, methods of fertilization, and egg inoculation.

(d) Larval rearing: We developed practical methods of larval rearing to the stage where fish could be stocked into growout tanks or ponds.

(e) Growout: We conducted growout experiments in a variety of tank and pond conditions, where the effects of water temperature, stocking densities, water depth, and water exchange rates were assessed (Figure 20).

Figure 20. Catfish, harvested from tank growout experiment, placed in wire-mesh cage to await weighing.

Importance to Users

The results of our research were extended to the private sector through workshops in 1986 and 1987 in cooperation with the UH Sea Grant College Program, the State Agriculture Department Program, and the Mariculture Research and Training Center of the UH, through the production of a 20-minute videotape, and through individual contacts with fish farmers. These extension efforts were successful in improving culture techniques on the one farm that had been producing *C. fuscus* before our work began, as well as providing technical information for 3 or 4 other farmers to begin production of this species. While there are still problems with the culture of this species in Hawaii, we have successfully removed the principal obstacles for its culture and created the necessary information base for production to increase many fold. The role of the University of Hawaii in providing these improvements is well recognized by the private sector.

Management Strategies to Prevent Delayed Puberty in Cattle in Subtropical Areas

Principal Investigators:
D. Vincent, Dept. of Animal Sciences, UH
C. Weems, Dept. of Animal Sciences, UH

Nature of Project

This project is designed to develop strategies to prevent delayed puberty in beef heifers raised in Hawaii. The problem is that heifers in Hawaii are not normally raised to give birth to their first calf at two years of age. The economics of beef cattle production strongly suggest that it is important to calve at two years. In Hawaii, cattle often do not have their first calf until three years of age, therefore the cost of raising an unproductive animal is increased. The project is designed to test a method of inducing puberty in beef heifers using a synthetic progestogen. In addition, the method is being tested on heifers on different grazing systems, i.e., continuous grazing, grazing on grass–legume combinations, and on intensive grazing systems, all typical of Hawaii cattle production. Breed effects of heifers are also being examined.

Major Achievements

In the second year of the trial, heifers were treated with Norgestomet implants at a very young age, i.e., 10–12 months, to determine if these animals would successfully respond to the synthetic progestogen by coming into heat and by becoming pregnant. Heifers were classified by breed groups, i.e., Bos taurus cattle (Hereford, Angus breeds); Bos indicus cattle (Santa Gertrudis, Brahman breeds) or the Mealani Synthetic Line (a synthetic line of cattle developed at the Mealani Research Station, Kamuela, HI). The Mealani Synthetic Line is a mixture of 6 breeds developed to be adaptable to Hawaii's many environmental conditions. The major achievement of this experiment was that heifers of the Mealani Synthetic Line responded to the treatment (underwent puberty and became pregnant) in greater numbers than either Bos taurus or Bos indicus breed groups.

Importance to Users

The importance of this trial is that heifers in Hawaii, raised on grass or grass–legume combinations, will respond to this treatment, even at very young ages, and become pregnant. Some breed groups may respond better than others. Ranchers now have an additional tool that can be used to induce puberty in beef heifers and to help synchronize reproductive cycles in their cattle. This should help them reach the elusive goal of having their heifers calve for the first time at two years of age.

The Molecular Basis of Thermotolerance in Tropical Plants

Principal Investigator:
H. M. Harrington, Dept. of Plant Molecular Physiology, UH

Nature of Project

All biological organisms, including plants and cultured plant cells, undergo what is termed the heat shock response (HSR) when subjected to brief periods of moderately warm temperature treatment. The HSR, first manifest on the molecular level, is characterized by the rapid cessation of most normal protein synthesis through both transcriptional and translational regulation. Heat shock results in new gene transcription and the synthesis of new heat shock proteins (HSPs) that are thought to provide some beneficial protective effect against otherwise lethal temperature treatment. Although much information is available relative to gene regulation and the basic characteristics of HSPs in plants, little data are available on the function of HSPs. This project employs a comparative physiology and biochemistry approach using cell cultures of a tropical plant (sugarcane) and a temperate plant (tobacco). These studies are focused on the identification of specific HSPs and the determination of their function during the development of thermotolerance.

Major Achievements

Characterization of the physiological parameters of the heat shock response in sugarcane has been completed. Briefly, these results indicate that sugarcane cells require a minimum heat shock of at least 30 minutes at 36C in order to develop tolerance to otherwise nonpermissive heat stress (7 minutes at 54C). Our results also indicate that temperatures in the range of 36 to 38C are most effective in inducing thermotolerance. Temperatures above or below the temperature optimum are ineffective in inducing the heat shock response. However, no heat shock treatment in the range of 32 to 42C (2 hr) causes any permanent damage to the cells as measured by the ability to maintain continued growth.

The characterization of radiolabeled amino acid incorporation into HSPs reveals that optimum HSP synthesis occurs in the 36 to 38C range (Figure 21). Temperatures below the temperature optimum are ineffective in inducing the synthesis of several low molecular weight HSPs. Alternatively, temperatures in excess of the optimum specifically inhibit the synthesis of higher molecular weight proteins such as HSP70 and HSP90. The failure to synthesize these two groups of proteins is undoubtedly the reason for the observed physiological temperature optimum of 36 to 38C for induction of the HSR.

The use of a cultured cell system has allowed us to determine very precisely the time course of HSP synthesis in pulse labeling experiments and to determine the time course of HSP turnover. The results of these studies indicate that a number of HSPs are synthesized within minutes after onset of elevated temperature. A complex of low molecular weight HSPs are not synthesized until 30 to 40 minutes after initiation of heat shock, however. The appearance of these proteins corresponds to the minimum heat shock time required for the induction of thermotolerance mentioned above. The difference in induction time for the synthesis of the low molecular weight complex (LMWC) HSPs relative to other HSPs suggests that some feature in the transcriptional/translational pathway leading to the synthesis of these HSPs is regulated differently. Analysis of HSP turnover indicates that the pool of LMWC HSPs appears to have a relatively short half-life, with these HSPs completely disappearing within 42 hr after heat shock. Parallel physiological experiments indicate that the cells also lose the ability to withstand nonpermissive heat stress at 54C during this same time frame. High resolution two-dimensional gel electrophoresis reveals that the complex consists of four similar polypeptides in the 17–18 kD molecular weight range. Based on the apparent absolute requirement for this complex of low molecular weight proteins, we are purifying and characterizing the components and are attempting to clone the genes for these HSPs to facilitate functional analysis and the determination of the molecular basis for the apparent differential regulation of LMWC HSP synthesis.

Importance to Users

Results obtained in these studies suggest that the basic regulatory features of normal and heat shock protein synthesis in sugarcane cells may be different from other systems. In all other biological systems examined thus far, the synthesis of most normal proteins is turned off through transcriptional and translational regulation during heat shock. Analysis of polysome profiles from sugarcane cells demonstrates that heat shock has little effect on the distribution of polysome size, indicating unchanged rates of protein synthesis. The results of *in vivo* labeling experiments have been confirmed by *in vitro* translation experiments indicating that both control and heat shock mRNAs are present on the polysomes isolated from heat-shocked sugarcane. These results are consistent with the idea that sugarcane continues to produce a normal complement of constitutive proteins during elevated temperature stress. In contrast, tobacco cells that fail to synthesize most constitutive protein during heat shock exhibit drastically altered (reduced) polysome profiles during heat shock. *In vivo* labeling of HSPs indicates that protein synthesis is reduced by 60 to 80% by heat shock. *In vitro* translation experiments using tobacco polysomes

or mRNAs also indicate that both control and heat shock mRNAs are present on heat shock polysomes. Thus, our results suggest that the continuation of normal protein synthesis in sugarcane may be due to the lack of some factor(s) that control(s) translation at the elongation level.

The ability to synthesize constitutive or normal proteins during elevated temperature stress may provide a competitive ecological advantage for sugarcane, allowing rapid growth and efficient productivity in tropical climates. If so, this feature may well provide important clues as to the success of present day tropical plants. The elucidation of the molecular features of the regulatory mechanisms which control protein synthesis under heat shock conditions may lead to the identification of key genes and gene products involved in the thermotolerance. These genes might be used to develop high-temperature-tolerant varieties through molecular biological approaches.

A portion of the tobacco research indicates that certain of the HSPs appear to bind to the calcium-dependent regulator, calmodulin. Our evidence indicates that several HSPs bind to a variety of calmodulins in a calcium-dependent manner. These data suggest that these proteins are, in fact, *in vivo* targets for calmodulin. Since calmodulin is known to act through a limited number of possible enzyme mechanisms, the probability is quite good that we will be able to assign a function to one or more of these HSPs. To this end, we have purified and characterized the binding characteristics of the binding proteins and are currently amassing enough of the polypeptides to obtain sequence data and to make antibodies. With this information we will be able to make genetic probes to screen a cDNA library for clones containing coding sequences for these HSPs. The sequence of the gene or protein may then be used to probe existing genetic/protein data bases to obtain clues as to the identity of the HSPs. These studies could well provide important insight into the regulation of the HSR as well as other metabolic events that are regulated by calcium.

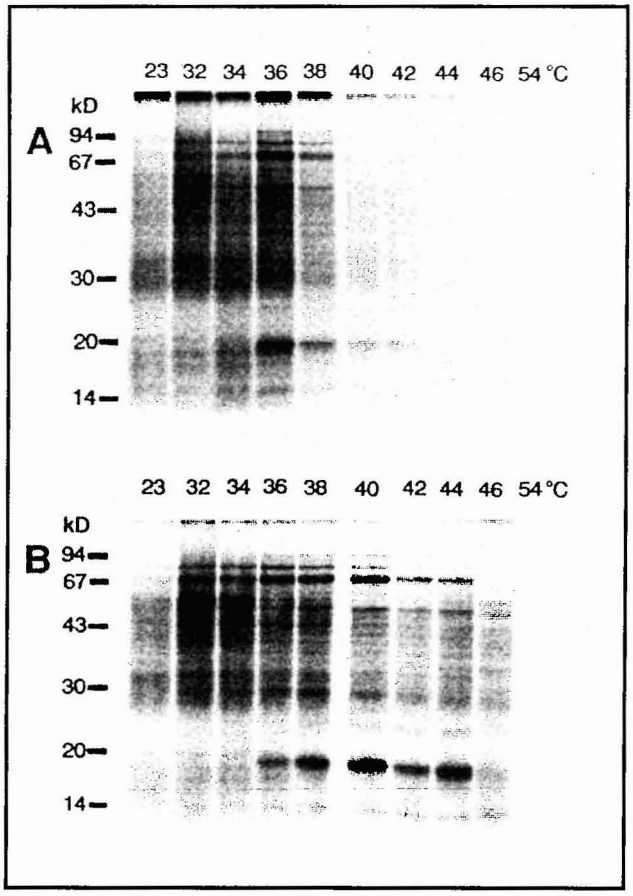


Figure 21: Effect of heat shock temperature on incorporation of labeled amino acids into protein. Cells were labeled for two hours and extracted. Proteins in the extract were separated by SDS-PAGE (12.5% gels). The radioactive proteins were visualized by flourography.

Panel A: **Each lane was loaded with equal amounts of total protein (20μg/lane).**

Panel B: **Each lane was loaded with equal amounts of radioactive protein (30,000 cpm/lane). Positions of molecular weight markers indicated in kD.**

Promoting Flowering and Dwarfing of Winged Bean with Plant Growth Regulators

Principal Investigator:
C. Lee, University of Guam

Nature of Project

It has been shown that naturally-occurring plant hormones play an important role in the process of flower bud induction and control of plant size. Synthetic plant growth regulators have been developed that will induce flowering at the desired time and reduce plant size on many agricultural crops. No work has been reported on the application of plant growth regulators for flower induction and dwarfing of winged bean. The objectives of this project are: (a) to assess the possibilities of year-round production of winged bean through flower induction, and increasing the number of flowers and pod sets by the application of growth regulators, (b) to evaluate the use of plant growth regulators for dwarfing so as to reduce labor and material costs of staking in the production of winged bean, and (c) to study the effect of plant growth regulators on the quality of winged bean pods and seeds.

Major Achievements

Experiments have been conducted to promote flowering and dwarfing of winged bean by the application of selective growth regulators. The optimum rates and effective growth regulators for the production of winged bean have been determined. A significant increase in number of fresh pods and yield of fresh pods resulted from the application of growth regulators such as 2,3,5–triiodobenzoic acid at 30 or 45 ppm, or (B–naphthoxy) acetic acid at 50 or 75 ppm, or (2–chloroethyl) trimethyl ammonium chloride at 50 or 75 ppm, or Spray–N–Grow at 3000 ppm.

The plant height was significantly reduced up to about 8 weeks after planting by the application of 2,3,5–triiodobenzoic acid at 30, 45, 60 ppm or (B–naphthoxy) acetic acid at 75 or 100 ppm, while the plant height was significantly reduced only up to 2 weeks after planting by the application of Spray–N–Grow at 3000 or 4000 ppm.

Importance to Users

This research has indicated that selective growth regulators play an important role in the process of flower bud induction, control of plant size and increased production of winged bean. People in the hot, humid, tropical areas of the world need better sources of protein from plants. The soybean, the world's premier protein crop, grows well mainly in temperate climates. Winged bean seeds virtually duplicate soybean in composition and nutritional value, therefore winged bean has great potential to supply protein needs in the tropics.

The possibility of year-round production of winged bean can be achieved by foliar application at early seedling stages of the selective growth regulators.

Characterization of the Maize Genome

Principal Investigator:
J. I. Stiles, Dept. of Plant Molecular Physiology, UH

Nature of Project

We are cloning and studying maize genes involved in determining agriculturally important traits. Our approach is to identify specific genes by expression and complementation of specific mutations in yeast. To accomplish this we constructed a cloning vector capable of expressing foreign genes in yeast. Initially, genes coding for enzymes involved in amino acid biosynthesis are our prime targets. To test the system, we have successfully expressed a wheat seed storage protein. As yet, however, we have not been able to detect complementation of any mutations in yeast.

Major Achievements

This research focused on the development of a system, for expressing foreign genes in yeast. In collaboration with scientists at the USDA/ARS laboratory in Albany, California, we successfully expressed a member of the α-gliadin class of wheat seed storage proteins (Figure 22). α-gliadin is one of the wheat flour proteins that determines nutritional and baking quality of dough. It is also the causal agent in celiac disease. In addition, we have used this system to investigate various factors affecting the efficiency of expression of foreign genes in yeast. This will improve their production in genetically engineered yeast. The system developed is currently in use in several laboratories around the world.

Importance to Users

Because we now have the ability to produce α-gliadin, we will be able to use genetic engineering to produce variants with improved baking and nutritional quality. Furthermore, α-gliadin is a major causal agent in celiac disease, a genetically predisposed condition that leads to intolerance to wheat products in the diet. By applying genetic engineering to the protein we should be able to determine the features of the protein that induce this disease and engineer α-gliadin that can be tolerated by celiac patients. This project has recently been featured in "Agricultural Research" published by the USDA–ARS (volume 36, No. 6, June/July 1988, page 11). In addition,

a patent covering this genetically engineered yeast has been issued. This was the first genetically engineered organism patent application submitted by the USDA in the Western Region.

Our yeast system is in use in several laboratories around the world expressing a variety of different genes. One example, being done at the University of Hawaii, is the expression of a protein with insecticidal activity that may, in the future, be used as a bioinsecticide. This is ecologically beneficial because it reduces pesticide use and subsequent environmental contamination.

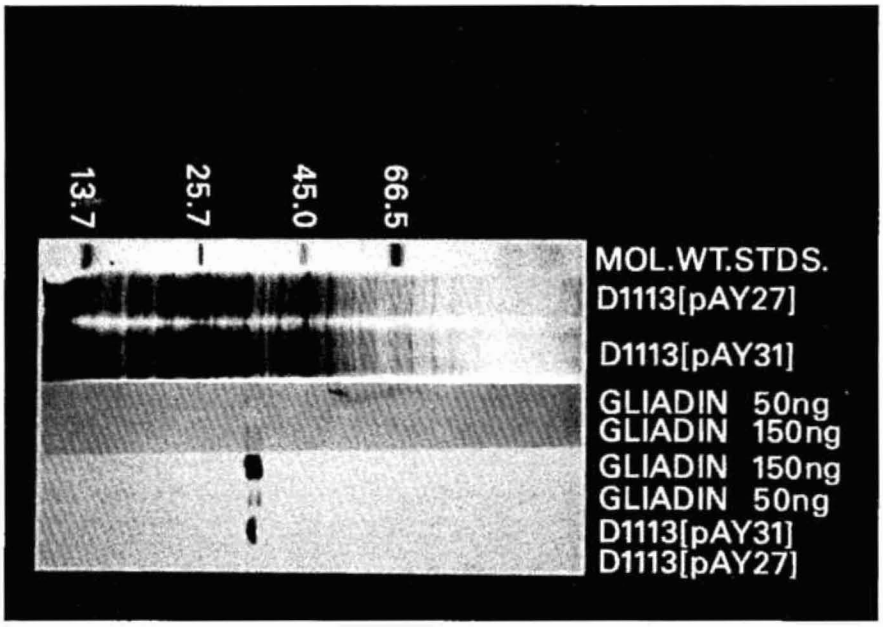


Figure 22: Analysis of proteins produced by a yeast strain containing the cloning vector and the vector with the α-gliadin gene. Lanes 1 through 5 from the left are a polyacrylamide gel strained for total protein. Lane 1 contains molecular weight standards, lanes 2 and 3 total protein from a yeast strain containing the cloning vector [pAY27] or the cloning vector with the α-gliadin gene [pAY31]. Lanes 4 and 5 contain α-gliadin purified from wheat flour. Lanes 6 through 9 are a "Western blot" for the gel shown in Lanes 1 through 5. The protein in the gel was transferred to a nitrocellulose membrane and probed with an antibody to α-gliadin. Lanes 6 and 7 are controls showing the reaction of the antibody with known amounts of α-gliadin purified from wheat flour (correspond to Lanes 4 and 5). Lane 8 shows the presence of α-gliadin in yeast containing the cloning vector with the α-gliadin gene, and Lane 9 shows that no α-gliadin is produced in yeast containing the cloning vector without the α-gliadin gene.

Micronesian Area Tropical Agriculture Data Base

Principal Investigator:
K. Carriveau, University of Guam

Nature of Project

The Micronesian Area Tropical Agriculture Data Base began as a cooperative project of the College of Agriculture and Life Sciences and the Robert F. Kennedy Memorial Library. The goals of the project were to gather in one location all published and unpublished documents produced in or about Micronesia concerning tropical agriculture and related subjects and to provide bibliographic information retrieval and document dissemination services. In 1986, The Micronesian Area Research Center agreed to assume responsibility for continuing the project after expiration of the grant and to expand the model into a topically comprehensive access tool.

Major Achievements

One major problem facing researchers in Micronesia used to be timely access to information about agricultural research. The problem has been largely resolved as a result of this project.

The major producers of agricultural documents in the Micronesian region have been identified, and memoranda of understanding for automatic deposit of materials have been negotiated with and signed by the governors and/or ministries of Commonwealth of the Northern Mariana Islands, the Federated States of Micronesia, the Republic of Belau, and the Republic of the Marshall Islands. Bibliographic databases such as AGRICOLA and BIOSIS were searched routinely for the identification of published materials about Micronesia as the basis for future acquisitions.

Computerized access to bibliographic information was provided to agriculturists both in inquiry and batch modes. The center used IBM's SQL/Data System as the bibliographic information management system. The system's Interactive Structured Query Language was specifically designed for people who have little or no knowledge of computers. The Micronesian Area Tropical Agriculture Data Base (MATADB) was designed to provide timely access to comprehensive bibliographic information and is intended to supplement rather than supplant existing resources, e.g., AGRICOLA. Each record is composed of seven columns, i.e., AUTHOR, TITLE, SOURCE, DOCNO (document number), CALLNO (call number), and KWI (key word index/abstract) The key word abstract is a composite of fixed language subject headings taken from the *Thesaurus of Key Words* and natural language key words abstracted from the text of the document itself. A user can request a bibliography of references by author, by words within a title, by source, or by subject. More sophisticated searches can be done by using Boolean logic search strategies.

The acquisition of materials was through purchases, site visitations, and automatic deposits. Once acquired, each item was indexed and abstracted for entry into MATADB. Monographs and serials were catalogued and incorporated into either MARC's Pacific Collection or the main library's collection for storage. Off-prints, reprints and unpublished materials were stored on specially designated shelves in the Micronesian Area Research Center (MARC).

A database called Agriculture Registry (AGREG) was developed to keep track of project participants. Its scope is similar to USDA's Current Research Information System (CRIS) and makes provision for the recording of a person's name, title, address, phone number, interests, and projects. The database was updated annually and served as a reference for identifying potential publications and for identifying appropriate audiences for the selective dissemination of information services.

A user's guide to SDI services was published and mailed to all project participants in order to facilitate the dissemination of information. The guide indicated how to formulate appropriate search strategies and provided the requisite forms for document photo duplication services. Once a search request was received, a "quick bibliography" was generated and sent not only to the requester but also to project participants who had a similar interest profile registered in AGREG. A "quick bibliography" of what had been published within the past year was also generated and was sent to all participating agencies in Micronesia.

Mini-workshops were conducted annually in order to promote the center's service capabilities. One workshop was open to the public and was presented as a Micronesian Area Research Center Seminar. Scientists and other users have been guaranteed that tailor-made bibliographies would be produced within 24 hours for off-line search requests or instantaneously through on-line searching.

Importance to Users

The center is now able to exchange bibliographic data with similar institutions either on tape or in hard copy. Informal working relationships have been made with the University of Hawaii's Pacific Collection, the Pacific Basin Development Council, the Institute of Pacific Islands Forestry, the University of the South Pacific's Pacific Information Center and the South Pacific Commission to share bibliographic data and/or publications. More formal working relationships have

been established through memoranda of understanding with the Ministry of Agriculture & Lands, Solomon Island Government; the Department of Agriculture, Kingdom of Tonga; the Ministry of Natural Resources Development, Kiribati; and the Institute of Natural Resources, University of the South Pacific. Interest in this area would seem to indicate that a Pacific region bibliographic network is feasible, but actualization of such a network may take some time to accomplish.

The University of Guam has committed resources to continue the work. The Micronesian Area Research Center recognizes the applicability of the model to other disciplines and has already committed funds to expand MATADB's scope to include all life sciences.

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